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AN EXPERIMENTAL STUDY OF THE GERMINATION OF WHEAT SEED UNDER WATER, AS RELATED TO TEMPERATURE AND AERATION*

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(WITH FIVE FIGURES)

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* Botanical contribution from the Johns Hopkins University, no. 111.

Introduction

The problem

The environmental complex operates conjointly with the internal characteristics of an organism to control physiological activity. The number of kinds of environmental influences that may act on a living thing is of course very large; it may indeed be regarded as infinite, for it includes, among other things, not only all the kinds of molecules and ions that may possibly act to alter the course of physiological activity in any way, but also as many partial ranges of radiation frequency as one chooses to consider. Each kind of influence may act with any number of degrees of intensity and intensity may fluctuate in any number of ways in the period of the life of the organism or throughout any developmental phase. Also, the time intervals considered in studying developmental changes may have any number of different lengths within the life period. Finally, the complex of all the environmental conditions that influence an organism for any time period may have any number of physiological values or capacities because of differences in the manner in which the component influences are combined or are concomitant.

Starting with a given set of internal conditions, as those of a seed placed in culture for germination, the course of development, or whether development shall occur at all, must be considered as determined by the environmental complex and the physiological capacity of the seed operating together. To appreciate the behavior of a germinating seed it is consequently necessary to take into account not only the whole of the influential complex acting from outside the seed but also the capacity of the seed to respond to or to withstand environmental influences. For a given set of internal conditions (kind of seed, health, vigor, etc.) different response patterns are brought into being for different combinations of effective environmental conditions and their various intensities; provided, of course, that the environmental differences considered are sufficiently great and prevail for sufficiently long periods of time to show noticeable corresponding differences in the behavior of the awakening organism.

To approach an experimental analysis of the conditional control of any kind of organism the problem needs to be attacked by the employment of relatively simple systems—the simpler the more promising—and this statement applies to the kind of organisms to be studied as well as to the environmental complexes that are to be employed. It is also desirable, in the beginning, to study physiological processes that lend themselves most readily to measurement and to comparison with respect to process rates and their end results. The general nature of the relations between organisms and their environments has recently been discussed from this view-

point by LIVINGSTON (**12**). Somewhat the same viewpoint was in part taken by F. F. BLACKMAN (**3**), in his classic discussion of environmental optima and limiting conditions.

Most students of the environmental relations of organisms have dealt with systems far too complicated for first steps in such analyses. The problems have in many instances involved too many experimental variables and many of these have failed to lend themselves to adequate numerical description. It has also occurred in many cases that the background variables (those not intended to differ in intensity from test to test) were not described sufficiently well to permit satisfactory approach to duplication if an experiment were to be repeated. These conditions of the experimental background might frequently differ from experiment to experiment and might fluctuate during the experiment periods in unknown and consequently unreproducible ways. Also, the several different intensities of the experimental variables themselves (the ones that are intended to differ in intensity from test to test) have been chosen in many instances so as not to represent adequately the whole range of intensities dealt with. To illustrate: only a few different maintained temperatures might be employed in a study of the temperature relations of a physiological process, when many additional temperatures would be needed to supply the points necessary for the construction of the ordinary temperature curve; the non-temperature conditions of the background might be inadequately described and the length of experiment period might not be chosen so as to bring out the time relations in a consistent manner.

The main purpose of the study to be reported in this paper was to attack a relatively simple set of physiological relations by use of a comparatively simple set of variables. The plant material chosen was a stock of wheat seed, whose germination capacity was to be studied in relation to various sets of environmental conditions. Resting seeds are relatively simple organisms in many respects, at least they are much simpler than seedlings or plants in later developmental phases. Wheat seed is known to retain its viability or capacity for germination without great alteration for rather long periods of time and the variability of a lot of pure-line wheat seed only a year or two old has been found to be not nearly so troublesome to the experimenter as is the variability of lots of some other kinds of seed. Although seed germination is a complex process, yet it is much simpler and more easily measured than subsequent phases of plant development and an end point for the germination phase may be chosen so as to be easily identified. Of course it is clear that the farther the developmental process is allowed to go the more complicated must be the environmental relations, and consequently this study dealt with the process of germination only, from the beginning of soaking to the bursting of the seedcoat.

As to environmental conditions, it is of course necessary that all essential ones for the process studied shall be represented. For the germination of wheat seed these are comparatively few. Light is not essential and the very complicated question of light relations is set aside at once by limiting the problem to germination as it occurs in darkness. Water relations are readily dealt with by having the seeds always under dilute nutrient solution of a 3-salt type, which is very simple in comparison with many other media suitable for seed germination. While wheat seed does not germinate well in distilled water, different proportions of the nutrient salts in a dilute SHIVE solution appear to be without marked influence on germination and the growth of very young seedlings, as was pointed out by GERICKE (**6**). Maintained temperatures are rather easily arranged in experimental studies that do not involve light. Letting the seeds germinate always under weak aqueous solution not only simplifies the question of water relations, as has been said, but it also simplifies the question of the influence of aeration. Natural aeration in a stagnant solution culture is not very vigorous and different degrees of aeration (including stirring of the solution) may readily be arranged by means of a bubbling air stream.

The duration factor, which is always troublesome in experimentation on environmental influence, was simplified by having the longest experiment period only 24 hours, which markedly limited the possible influence of unknown changes in the medium as well as the extent of fundamental changes in the organisms during an experiment. This question of the influence of alteration in the influential conditions is of paramount importance and it would surely require serious attention if the seedlings were allowed to advance beyond the bursting of the seedcoat or if the experiment periods were much longer than the longest one employed in this study. By the use of three experiment periods shorter than 24 hours data were secured which may be useful in case later studies make the 24-hour period appear too long for the other features of the experimentation, especially with reference to unknown progressive changes in the nutrient medium—such as absorption of salts or ions and leaching or excretion of material from the seeds.

Seed germination was chosen as the subject for this study primarily because it constitutes a readily measured physiological process reduced nearly to its lowest terms of complexity. But there are other good reasons for further experimental study of the viability of a lot of seed. Practical methods for seed testing must be based on the principles to be brought out by such experimentation as this and the more we know of germination in general the more rapidly can seed-testing procedure be improved.

Many experimental studies on the relations of more advanced developmental phases of plants involve the use of plant material secured from

seeds—indeed, for higher plants experimental material must be derived from seeds unless cuttings or other means of vegetative propagation are employed. And one of the main desiderata for any kind of experimentation with plants is that the different individuals of the stock of plants used should be as nearly alike as possible. Whenever seedlings or subsequent stages of development are to be employed it is necessary that serious attention be given to the conditions that prevail during seed germination and it appears that one of the most promising ways to procure a lot of reasonably similar plants is to carry out selections based on germination and the very early phases of seedling growth. Thus, the results of a study such as the one here reported may be valuable in a fundamental and very practical way, as furnishing bases for many kinds of experimental study of more advanced phases.

Because seed germination constitutes a conspicuous example of the awakening of plant organisms from a dormant condition, after all physiological processes have been at very low ebb for a long time, this kind of growth is itself of special importance in general physiology.

The present study was carried out at the Laboratory of Plant Physiology of the Johns Hopkins University, from the fall of 1929 to the spring of 1930. In the planning of the experimentation and in the assembling and presentation of the results the writer has been guided and helped by Professor BURTON E. LIVINGSTON, director of that Laboratory, to whose interest this paper is largely due.

EARLIER STUDIES ON SEED GERMINATION

Ever since the earliest days of botanical science seed germination has been studied, sometimes from the viewpoint of physiology but in most cases with regard to agricultural and horticultural practice, and the literature of this general subject is very extensive. Recent contributions on seed germination may be classified in two categories: (1) Studies undertaken chiefly with reference to seed testing, for comparing different lots of seed in regard to their various degrees of viability or germinative energy. To this group belongs the work of HARRINGTON (**8**) and WILSON (**27**). (2) Studies on seed dormancy and on special treatments by which the dormant period may be shortened. Here belongs the work of CROCKER (**4**), ATWOOD (**1**), HARRINGTON (**9**) and MORINAGA (**19**). Most of the more recent studies are more satisfactory than earlier ones, but the interaction of the organism and its environment deserves more thorough attention than has been given to it. Experimentation on seed germination has generally dealt with the behavior of the organisms in relation to rather limited ranges of artificially controlled or measured experimental conditions and little attention has been paid to the other variables, which are of course influential along with the experimental ones.

Several contributions to our knowledge of this subject should be mentioned here, attention being confined to the few papers that deal with germination from viewpoints more or less similar to that of the present study or that furnish information pertinent to the interpretation of the results to be reported in subsequent sections of this paper. In a study of optimal temperatures for seed germination, employing seeds of 18 different plant forms, a series of maintained temperatures and a number of different substrata, HARRINGTON (**8**) ascertained the temperature and the length of time best suited for testing the viability of the kinds of seed studied. The results show how different kinds of seed differed in promptness of germination and how different temperatures differed in their influence on different kinds of seed. These results are of course referred to the ranges of influential conditions that were included in HARRINGTON'S experiments, but it is impossible to gain a clear idea of his prevailing non-temperature conditions because these were not controlled in detail and their description could not be made as specific as would be necessary if these tests were to be repeated.

WILSON (**27**) studied the germination of wheat seed on moist plaster-of-Paris plates at different maintained temperatures, continuing each test till no further increase in the number of germinated seeds was observed. The number of days required for the earliest and for the latest germination was recorded in each instance. Some of his resulting data are shown below, for five different maintained temperatures.

	10°	15°	20°	25°	30°
Average germination percentage	93.5	96	94	90	78
Average no. of days required for earliest germination	5	4	2.5	2	1
Average no. of days required for latest germination	11	10	6.5	4	4

From these data WILSON concluded that the optimal temperature for germination was 15°, because that temperature gave the highest average germination percentage at the end of the experiment. For 15° the final percentage was 96 and the period was 10 days, but nearly as high percentage values were obtained at temperatures of 10° (93.5 per cent. in 11 days), 20° (94 per cent. in 6.5 days) and the final percentage for 25° was of the same order of magnitude (90 per cent. in 4 days). In

studying these results it is surely permissible to consider 93.5, 96 and 94 as alike, and it seems clear that, when we consider the duration factor, 20° must be regarded as much more nearly the general temperature optimum than 15°. At any rate, it is clear from the tabulation just presented that any temperature between 10° and 25° might be expected to give nearly complete germination in a lot of seed like that used by WILSON.

MORINAGA has recently reported a series of studies on germination (**19, 20, 21**) and those devoted to germination under water are of special interest in connection with the present report. In each of these water experiments the seeds were on the bottom of a 100-ml. Erlenmeyer flask filled with distilled water and kept in a greenhouse at a temperature fluctuating between 15° and 35°, the water being renewed every two weeks. A seed was considered as having germinated as soon as the hypocotyl or plumule showed notable enlargement. Out of 78 plant forms tested, the seeds of 43 germinated under water and 18 of these showed no decided differences between germination in water and germination on wet filter-paper. For the remaining 25 germination was more complete or more rapid on wet paper. MORINAGA reported that his wheat seed was unable to germinate under water, thus agreeing with similar results secured by KRAUS, who found, according to MORINAGA, that his wheat seed did not develop under water farther than to the stage at which the coleoptile protruded. In some of MORINAGA's experiments wheat seeds, as well as seeds of some other kinds that failed to germinate under water, were able to do so when nearly pure oxygen replaced the air above the water. This apparent influence of aeration in connection with seed germination under water is surely important.

In one of MORINAGA's studies (**19**) 10 samples of 10 seeds each of white clover were tested under water and a similar series of samples was tested on moist filter paper in Petri dishes, at maintained temperatures of 5°, 10°, 15°, 22°, 27°, 32° and 38°. The germination percentages were recorded after 2, 6, and 10 days. The results show a number of remarkable things, some of which were pointed out by MORINAGA. When germinating under water the optimal temperature for this white-clover seed lay between 15° and 27° for all incubation periods studied, but the corresponding filter-paper tests gave a temperature optimum about 22° for a 2-day period and an optimal range of 10°–22° for the longer periods. There was thus a broadening of the optimal temperature range with longer time of incubation when the seeds were on filter paper, a phenomenon apparently related to the shifting of the optimal temperature shown by WILSON's data as well as by those of HAASIS, which will be referred to later on. Had MORINAGA chosen more and shorter time intervals he would probably have obtained a notable downward shifting of the optimal temperature for his filter-paper cultures as the incubation period became longer. The minimal temperature

for germination, for both water and filter-paper cultures was about 5° for the 2-day period and for longer periods it was considerably lower. The maximal temperature for germination was not shown for any period with the seeds under water, being always well above 38°, but it was apparently not much above that temperature when the seeds were on wet filter paper. Furthermore, the rate at which MORINAGA's lot of white-clover seed germinated in water is much more rapid than the corresponding rate for cultures on filter paper.

By studying the germination of seeds under water, MORINAGA certainly advanced a great step beyond earlier workers in this field. His moisture and aeration conditions were thus much more definitely specified than is possible with other methods of treatment. Since his incubation periods were rather long it might have been better to renew the water oftener than at 2-week intervals, but each time the water is renewed in such cultures the seeds are rather thoroughly aerated for a short time. A continuous flow of the liquid medium might be valuable in such experiments, if it could be arranged, and that might care for the maintenance of aeration conditions as well as of other solution characteristics.

MORINAGA suggested that the differences which he observed between germination in distilled water and on wet paper might be related to oxygen supply and the accumulation of toxic products of respiration. Such products might not accumulate so much in seeds surrounded by liquid medium as in seeds on wet paper; for the liquid medium acts to remove excreted material from the immediate surroundings. The so-called poison action of distilled water may perhaps deserve consideration in this connection. It has been studied by many writers, among them LOCKE (**16**), RINGER (**23**), OSTERHOUT (**22**), LIVINGSTON, *et al.* (**14**), TRUE (**26**), MERRILL (**18**) and GERICKE (**6**). There seems to be no doubt that a weak nutrient solution is, in general, more satisfactory as culture medium than distilled water is. Distilled water is never quite pure and it may influence an organism in contact with it by supplying the absorbing cells with influential ions or molecules in extreme dilution, as Cu or Zn or organic substances that distill with steam. This possibility is difficult to avoid. Distilled water also differs from a nutrient solution in that it furnishes no considerable diffusion tension of the essential inorganic ions and therefore generally permits more loss of substances from the organism than would occur if a good nutrient solution were employed. Finally, ions and molecules leached from the absorbing cells may accumulate in the medium and, with or without modification, these may subsequently exert considerable influences upon the organism. Leaching appears to occur more rapidly when seeds are under distilled water than when they are under a fairly well balanced nutrient solution.

HAASIS (7) studied the germination of seeds of several species of coniferous tree with special reference to the influence of incubation temperature and length of the incubation period. He emphasized the importance of defining or specifying all the influential background conditions of an experiment, although these do not enter directly into consideration as experimental variables. He also emphasized in a new way the importance of the time factor in physiological processes. His results with pitch pine seed are specially interesting in the present connection. Employing an extensive series of maintained temperatures ranging from 16° to 57° and incubation periods ranging in length from 6 or 7 hours to 14 days, he observed that the optimal temperature for the germination of his pitch pine seed shifted from about 47° (for a 1-day period) to about 23° (for a 10-day period) apparently remaining unchanged for more prolonged incubation. With incubation periods of intermediate length there was evidence of two very different optimal temperatures, one at about 31° and the other at about 43°. This important observation would have escaped attention had the incubation periods of about 2 or 3 days been omitted from the plan of HAASIS's study. The same writer also emphasized for the first time the possibility of using a series of different germination treatments for the purpose of defining and sorting out several physiologically different classes of seeds from a given lot. HAASIS's technique did not allow very definite specification with regard to moisture and aeration conditions. All of his seeds were treated with hydroxy-mercuri-chloro-phenol ("semesan") to avoid mold growth. His experimentation was unusually well planned and his data for pitch pine seed are exceptionally complete and generally very consistent. Many interesting points are brought out in his discussion.

Methods and procedure

THE MAINTAINED TEMPERATURES

For maintaining the desired temperatures the series of seven chambers described by LIVINGSTON and FAWCETT (15) was employed. The apparatus stands in one of the greenhouse rooms of the Laboratory of Plant Physiology of the Johns Hopkins University. The chambers are vertical cylinders 28 cm. in diameter and 43 cm. high, each with a water jacket and a stirrer to keep the water in slow rotation. The whole series is well insulated from the outside air by means of packed ox hair and wood and each chamber has a removable lid made of wood and cork. Adjacent water jackets are separated from each other by vertical partitions of galvanized and asphalted sheet iron. Water does not move from one jacket to the next but heat is transferred through the partitions being supplied at one end of the series by means of a thermostatically controlled electric heater and removed at the other end by means of an electrically driven and auto-

matically controlled mechanical refrigeration machine. A thermal gradient is thus maintained between the two ends of the series and each of the seven chambers has its own maintained temperature, which fluctuated, in the present study, within a range of about plus or minus 1° C. Proceeding from the warm to the cool end of the row, each chamber is cooler than the next succeeding one. By suitable adjustment of the heating and refrigeration controls different series of maintained temperatures may be secured. For this study the temperatures employed were 12°, 19°, 24°, 30°, 35°, 40° and 45° C. The continuous, slow rotation of the jacket water around each chamber gives to all sides of the chamber practically the same temperature; the lower part of the air mass enclosed in a chamber was found to be generally a little cooler than the upper part but the difference between bottom and top was not over 2°. The culture flasks stood upright at the bottom of the chambers, all at the same level, accompanied by a thermometer or a thermograph, as space allowed.

Germination flasks and accessories

Erlenmeyer flasks, of "Pyrex chemically resistant" glass 15 cm. high and with bases 9 cm. in diameter, were used in these germination tests, each flask containing 100 ml. of nutrient solution. The solution was 2.3 cm. deep and the diameter of its circular free surface was 8.5 cm., where it was in contact with the air. For the tests with flowing air each flask was closed by means of a 2-hole rubber stopper bearing an inlet and an outlet tube (inner diameter, about 5 mm.), the inlet extending downward nearly to the flask bottom while the outlet terminated just below the stopper. Three centimeters above the stopper each tube was bent at a right angle, with the horizontal arm 3 cm. long. In operation, each inlet tube was joined to the air-supply tube from its own wash bottle (in the same chamber with the germination flask) and all outlet tubes were joined to a line of tubing leading to an aspirator. All tubing was of glass, with short couplings of rubber tubing. In most of the experiments air from the greenhouse was continuously bubbled through the nutrient solution in each germination flask. At the end of each test the germination flasks were washed with cleaning fluid, thoroughly rinsed with distilled water and allowed to dry by draining before being used again.

Aeration of cultures

For each germination flask with air flow there were two wash bottles of water, so connected that the air stream entering the flask had bubbled through them in series. The first of these (outside of the chamber) was a 200-ml. bottle provided with rubber stopper and two tubes, for inlet and outlet. The inlet tube opened into the greenhouse and the outlet led to the second wash bottle (inside the chamber). The latter was a 1-quart

"Mason" jar, with rubber stopper bearing inlet tube and outlet tube, the inlet leading from the first wash bottle while the outlet led to the flask. The first of these bottles was half filled with water and served primarily as a telltale, used in ascertaining from time to time the rate of air flow in terms of the rate of bubbling; it also served to increase the humidity of the air stream while the latter was still at greenhouse temperature. The second wash bottle (within the chamber) was about half full of water, at the maintained temperature of its chamber. This bottle served to bring the air stream to the desired temperature before it reached the germination flask. The air entering the flask was found always to have the temperature of the chamber in which the flask stood, even with the most rapid rate of air flow used and with the lowest and highest temperatures employed. In passing through the second wash bottle the flowing air was automatically brought to approximate vapor-pressure equilibrium with the dilute nutrient solution in the flask, so that evaporation or condensation in the flask was at a minimum.

The tube leading from each flask was provided with a special orifice, just outside the temperature chamber, for the controlled application of suction from the suction tank—on the principle that rate of gas flow is determined by pressure gradient and orifice resistance. This device was similar to the one used by HUTCHINS (**10**) for a like purpose, consisting of a plug of cotton wool and pulverized kaolin suitably packed in a slightly conical tube. Each plug was adjusted, by the proper degree of packing, to give the desired rate of flow with the suction employed. To prevent excessive condensation of water in the plug, which might increase its resistance to air flow, HUTCHINS kept the plug temperature always somewhat higher than that of the entering gas, but the same end was attained in the present experiments by means of a drying tube with calcium chloride, inserted between flask and plug inside the chamber. Five different orifice settings were used, with air-flow rates of about 1, 3, 6, 15 and 30 liters per day, or 40, 125, 250, 625 and 1250 ml. per hour. The corresponding numbers of bubbles passing through the telltales were 4, 12, 24, 60 and 120 per minute. The orifices were nearly alike for all flasks in the same experiment and the rates of bubbling in the flasks were similar to those shown by their respective telltales. The discharge tubes all led out of the temperature chambers to the suction tank, a 5-gallon bottle with rubber stopper, inlet tube and outlet tube, the latter leading to a "Cenco-Harrington" filter pump attached to the greenhouse water supply.

The nutrient solution used was of very low vapor pressure, its osmotic value being about 1 atmosphere for 20°, and neither condensation nor evaporation of water was observed to have occurred in any of the flasks, even at the end of the longest experiment period and with the most rapid

rate of air flow and the highest and lowest temperatures employed. This indicates that the second wash bottle in the air line leading to each flask operated effectively as both temperature and humidity control for the air stream. The surface level of the solution in the flasks was consequently always about the same (about 2.3 cm. above the flask bottom) and the submerged seeds were always at practically the same depth. The volume of solution around and above the seeds altered slightly, of course, through absorption and swelling, but water absorption and swelling by such seeds as wheat are almost equal in respect to volume. It is interesting to note that a hundred seeds had absorbed about 2.5 ml. of water when swelling was complete and that the volume of a swollen seed was about twice as great as its original volume before soaking. The total volume of seeds and water decreased by about 0.4 ml. in the first 24 hrs. of soaking. There were apparently some very small gas-filled spaces in the dry seeds, which became filled with water as soaking and swelling proceeded.

The air stream continuously supplied oxygen to the solution across the bubble surfaces and the free surface of the liquid. Across these same surfaces it continuously removed carbon dioxide and any other volatile substances that may have escaped from the seeds. Also, the mechanical action of the bubbles kept the solution in circulation above the seeds and around them and the rate of stirring was determined by the rate of bubbling. It is of course impossible to study these three effects of the bubbling gas stream separately without elaborate technique, but they need to be borne in mind in connection with all experiments with air flow.

Cultures without air flow

Besides the cultures that were artifically aerated by means of the air stream, there were also corresponding cultures without artificial aeration. For these the procedure was the same as that just described excepting that the flask stoppers were without tubes. Aeration of the solution was limited to such interchange between flask and chamber as might take place through the two perforations in the stopper and such very slow mixing or stirring of the solution as might be due to natural convection.

The nutrient solution and its use

The culture medium used throughout these experiments was a 3-salt nutrient solution of a type used extensively by Shive (**24**), being solution R 3.3, S 3.3 of type I, as described in the "Plan" of the Committee on the Salt Requirements of Plants, of the U. S. National Research Council (**13**), with equimolar partial concentrations of the three salts and a total osmotic value of about 1 atmosphere at 20°. It contained per liter 0.0084 gram-mol. of each of the salts, KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$. No iron

was added. The salts used were of the Baker Chemical Company's "C.P., analyzed" grade. The water used was derived from a Barnstead still.

Single-salt stock solutions were prepared in a way similar to that followed by SHIVE, enough of each being prepared at the start to last throughout the whole study. The concentrations of the three stock solutions were: KH_2PO_4, 0.59 molar; $Ca(NO_3)_2$, 1.17 molar; $MgSO_4$, 1.01 molar.

Slight turbidity appeared in the KH_2PO_4 solution as originally made up and this was removed by filtering, the final concentration of the solution being ascertained by evaporating a sample to dryness, igniting the residue and weighing the latter as KPO_3—as recommended by TREADWELL and HALL (**25**, p. 612). The concentration of the $Ca(NO_3)_2$ solution was ascertained gravimetrically, by precipitating the calcium of a sample as oxalate, igniting the latter and weighing it as CaO—as recommended by the authors just mentioned (**25**, p. 81). The $MgSO_4$ solution was based on the weight of salt used, considering it as $MgSO_4 \cdot 7H_2O$, and no analysis of that solution was made. From the three single-salt solutions supplies of the 3-salt nutrient solution were made up in the usual way, as needed.

The nutrient solution must have altered considerably during an experiment period and it was presumably very different at the end from what it was at the start. Changes must have been brought about through absorption of water and salts by the seeds and through exudation of material from the seeds. After 24 hours at the highest temperatures used the culture solutions were noticeably turbid. The kind and amount of alteration that occurred in any flask must have been somehow a function of the combination of the variables involved, which were by nature more or less interdependent, and any attempt to study the nature and course of such solution changes adequately would be difficult.

THE SEEDS USED AND THEIR PRELIMINARY TREATMENT

The wheat seeds used in this study were of the "Nittany" variety, from a supply received from the Pennsylvania Agricultural Experiment Station in the fall of 1928. Some of this same stock had been used in MACK's study (**17**) of carbon-dioxide production and growth by young seedlings. No evidence has been encountered to suggest the occurrence of any alteration in the physiological characteristics of this lot of seed between the time it was received and the conclusion of the present study in the spring of 1930. It is therefore probably safe to suppose that MACK's results and those of the present study are comparable with respect to the characteristics of the seed used. The supply was stored in a burlap sack in an attic room, with temperature between 20° and about 25°. In September, 1929, weevils appeared and the entire supply was treated at that time with carbon-disulphide vapor, after which no more weevils were observed.

Sample tests made before and after this treatment showed no influence of the treatment upon the subsequent behavior of the seeds; their viability was apparently not altered at all.

The seeds for each experiment were selected with respect to specific gravity and general appearance just before they were introduced into the germination flasks. About 30 minutes before the start of an experiment approximately twice as many seeds as would be required (about 150 ml. of dry seeds) were placed in a glass pan 15 cm. in diameter and 3 cm. high and enough nutrient solution was added to just fill the pan. After vigorous stirring for a few seconds all floating seeds, comprising about 5 per cent. of the total number, were removed and the remaining seeds (those of greater specific gravity than the nutrient solution) were spread out on filter paper, from which they were counted out in 100-seed samples into 100-ml. Florence flasks, as many flasks being used as there were samples. All broken or otherwise apparently unusual seeds were rejected.

The selected seed samples were then transferred to the several germination flasks in the maintained-temperature chambers. These flasks had been prepared about an hour earlier, charged with nutrient solution, distributed among the seven temperature chambers and made ready for starting the air streams. When the seeds were introduced, the nutrient solution in each germination flask had already attained the temperature of the chamber in which it stood and the seeds must have acquired that temperature very quickly after their introduction. It required only about a minute to set the stopper in each of the germination flasks and start the air stream. The seven cultures of an experiment were started one after another in the increasing order of their temperatures and about 2 minutes elapsed in each instance. Thus the culture in the second chamber was started 2 minutes later than the one in the first chamber, the culture in the third chamber was started 4 minutes later than the one in the first, and so on, the culture in the seventh chamber being started about 12 minutes later than that in the first.

The experiment periods

How many of the hundred seeds in any test were found to have germinated at the end of an experiment period must have depended in general on the length of the period as well as on the maintained temperature, the aeration treatment and the background conditions, the last-mentioned complex being initially alike for all experiments. The four different time periods employed in this study were 6, 12, 18 and 24 hours in length. The shorter periods were not simply observation intervals in the longest period but period length was treated as a definite experimental variable, there being a separate set of experiments for each of the four different periods. At the end of each experiment the germination flasks were re-

moved from the temperature chambers in the same order as that followed in starting the cultures. The solution was decanted and the seeds were spread out in a Petri dish, from which those that had germinated were counted to give the germination percentages. These operations required about 7 minutes in each instance; consequently the culture for 19° was observed about 7 minutes later than that for 12°, etc., and the culture for 45° was observed about 42 minutes later than that for 12°. It is evident that the actual experiment periods were not precisely the same for the different cultures of an experiment, the actual time being generally a little longer than the schedule time, with the difference somewhat greater for higher than for lower temperatures. In no instance, however, did the difference amount to more than about 30 minutes, and the nature of this study does not warrant the application of special corrections for these small discrepancies.

It may be noted that earlier students of seed germination have generally employed observation intervals as parts of a longer experiment period and that the method used in this study is comparatively new for this sort of investigation. The essential differences between these two methods of securing information on the influence exerted by the duration factor in studies of this kind deserves some attention. Let it be desired to secure experimental values for the germination percentage of a lot of seed for time periods of 6, 12, 18 and 24 hours, the environmental complex being considered as the same for all four durations, or at least for the beginnings of all tests. According to the usual procedure (as that followed in HAASIS's recent study, for instance) the cultures would all be continued for 24 hours, the number of seedlings produced at the end of 6, 12, 18 and 24 hours being ascertained by observations at the ends of the several 6-hr. intervals. After the first, second and third observations the ungerminated seeds would be returned to the standard environmental complex for the next interval. The exposure for 12 hours would consequently have to be interrupted at the end of the sixth hour, the exposure for 18 hours would have to be interrupted at the end of the sixth hour and at the end of the twelfth, and the 24-hour exposure would have to be interrupted three times, at the ends of the sixth, twelfth and eighteenth hours. Such interruptions should introduce corresponding fluctuations in the environmental complex, which usually can not be satisfactorily maintained through the short time periods requisite for observation, and these environmental fluctuations are apt to be very difficult to measure and to take into account when the experimental results come to be compared and interpreted. Common practice has been to regard the possible influence of these fluctuations as negligible, which is scarcely permissible unless suitable preliminary tests have shown this supposition to be legitimate.

When duration is treated simply as one of the experimental variables, as was done in these experiments, no interruption of exposure needs to be introduced. No culture is disturbed till the end of its own test period and observations are made only when tests have been completed. So the environmental complex is maintained throughout the experiment period without any fluctuation due to observations, though of course it may fluctuate more or less for other reasons, just as it does between times of observation when the method of intervals is employed. In the present study the relations between duration of exposure and a given set of environmental features are shown by the behavior of four different seed samples, a separate sample of 100 selected seeds being used for each of the four different exposure periods. On the other hand, the commonly used method of observation intervals and interrupted exposures does allow the same individual sample of seed to be exposed for all of the different periods considered, an advantage that is not offered by the procedure followed in this study. Various features of experimental technique, as well as the pertinent logical considerations, will naturally need to be taken into account when it is to be decided which of these two methods for dealing with the duration factor is to be employed for a projected series of experiments.

The criteria of germination and the germination percentages

For the purposes of this study a seed was considered to have germinated when the white coleoptile became visible through rupture of the seed-coat. This is the earliest stage of germination that can be readily and definitely recognized by ordinary ocular observation. At the end of each experiment each culture of 100 seeds was examined, as has been said, to ascertain how many seeds had attained this stage, including those that had progressed somewhat farther, and that number was taken as the germination percentage. Each test was repeated four times and each of the final germination-percentage values is the average of five values derived from as many like tests.

The average germination percentage for any particular environmental-durational complex indicates, of course, approximately what fraction of the number of seeds actually tested were capable of germinating with the specified complex. For example, this lot of wheat seed was of such nature that 33 per cent. of the selected seeds germinated, on the average, in 12 hours if submerged for that period under about 2 cm. of the standard nutrient solution aerated by an air flow of 1 liter per day and maintained at a temperature of 30°. No other one of the 168 different treatments tested gave this index value and only two treatments gave corresponding index values having magnitudes between 29 and 37, inclusive. Such physiological characteristics of a lot of seed may well be as valuable as other character-

istics that have been employed in describing lots of seed, such as average size, average weight, average starch percentage, etc. If a lot of seed is to be described in terms of viability (that is, capacity to produce seedlings) physiological indices of this sort will surely prove to be not only useful but in many instances quite essential. The results of this study actually furnish 168 different viability indices for the lot of seed used, based on as many different treatments or environmental-durational complexes.

It is of course also true, in a sort of converse way, that the average germination percentages resulting from a consistent and adequately inclusive set of tests are efficiency indices of the several environmental-durational complexes to which they correspond; any result of measurement defines both the thing measured and the measuring device, the former with reference to the device and the latter with reference to the thing measured. One of the aims of this study was to find out how the lot of seed used would behave or perform with reference to a series of treatments but another aim was to find out what each of the several treatments would do to representative samples of this lot of seed. Either aspect implies the other. The average percentage values are consequently to be regarded as approximate measurements of the capacity or capability of this lot of seed to produce seedlings and at the same time they are approximate measurements of the capacities of the several environmental-durational complexes to permit or call forth the production of seedlings from samples of this lot of seed. In short, we enquire, what can this lot of seed do and what can these complexes do?

As to the significance of the average germination percentages, these refer, of course, to the lot of seed used in this study and to no other lot. Other lots of the same variety or of other varieties might be expected to exhibit different kinds of germination performance. The several averages also refer to the corresponding treatments, as has just been mentioned. With another nutrient solution, with other maintained temperatures than were actually tested, or with other aeration procedures, this lot of seed or any other lot of seed might have performed very differently.

How nearly the several 100-seed samples tested may have represented the stock of seed dealt with and how nearly alike the several samples were among themselves, are important questions best answered by a study of the consistency of the whole system of numerical results. The average percentages would of course have been somewhat more nearly representative of the lot of seed from which the samples were taken if the tests had been repeated a greater number of times or if each sample had included a larger number of individual seeds. The numerical values show that there was considerable variability among the five supposedly like tests, which may indicate either effective degrees of difference between the environmental complexes for the five tests or effective differences between the capacities of the five 100-seed samples; or both sorts of differences may have occurred

together, which is not unlikely. An examination of the average percentage values leads to the conclusion that they are in general remarkably consistent and that the forms of their graphs would not have been very different if more than five tests of a kind had been made or if more than a hundred seeds had been employed in each test.

If any reader is inclined to apply statistical methods to the average germination percentages the way is clear, for all the values are given in table I. It does not appear, however, that anything would be gained by such treatment of these data and the following discussions will be based on the general consistency of the averages, which is generally of a relatively high degree for such studies as this.

General plan of the experiments

It was planned to cover uniformly a definite range of environmental conditions and the entire series of experiments involved tests of all possible combinations of the 7 temperatures, the 6 sets of aeration conditions and the 4 period lengths. Each experiment included just 7 cultures, one for each temperature employed but all with the same aeration treatment and the same period of incubation. The entire series of experiments involved altogether 168 different tests, each with 100 seeds that had been selected by observation and with respect to specific gravity, as has been noted. Each of the 168 tests was made five times, making a total of 840 tests. The earlier experiments were conducted singly but in the latter part of the study two experiments were conducted simultaneously. There were no indications leading to any suspicion that the lot of seed used altered in any sensible way during the term of the present study, and the general consistency of the results indicates that the experimental technique was equally satisfactory for all experiments. As a matter of record, the dates of the routine experiments are added here.

No air flow	Aug. 30–Sept. 10; Oct. 8–14, 1929.
Flow of 30 l. per day	Aug. 30–Sept. 10; Oct. 2–13, 1929.
Flow of 3 l. per day	Oct. 13–Oct. 29, 1929.
Flow of 15 l. per day	Oct. 30–Nov. 11, 1929.
Flow of 6 l. per day	Nov. 12–Nov. 21, 1929.
Flow of 1 l. per day	Nov. 23–Dec. 2, 1929.

Besides the routine tests thus far described some special experiments were performed to gain information on questions that arose as the regular numerical results accumulated. A few of these will receive some attention after the routine experiments have been discussed.

Results of routine experiments

The average percentages and the primary graphs

All the percentage values from the routine experiments are presented in table I. The four main vertical sections of the table correspond to the four

TABLE I

GERMINATION PERCENTAGES FOR FIVE SEPARATE TESTS OF EACH ONE OF 168 DIFFERENT CONDITIONAL COMPLEXES, TOGETHER WITH THE AVERAGE PERCENTAGE FOR EACH COMPLEX

(FOR EACH FIVE LIKE TESTS THE MINIMUM AND THE MAXIMUM ARE IN ITALICS AND THE AVERAGE IS IN BOLDFACE TYPE)

	TEMPERATURE	FOR 6-HOUR PERIOD		FOR 12-HOUR PERIOD		FOR 18-HOUR PERIOD		FOR 24-HOUR PERIOD	
		PERCENTAGES FOR 5 TESTS	AVERAGE	PERCENTAGES FOR 5 TESTS	AVERAGE	PERCENTAGES FOR 5 TESTS	AVERAGE	PERCENTAGES FOR 5 TESTS	AVERAGE
Cultures	*deg. C.*		*per cent.*		*per cent.*		*per cent.*		*per cent.*
without air flow	12	3 3 *1* *8* 3	**4**	7 *8* *6* 7 7	**7**	*10* 12 12 *10* *15*	**12**	*10* 13 *14* *14* 13	**13**
	19	5 *4* *4* 7 *11*	**6**	*15* 9 10 *7* 13	**11**	17 *12* *21* 15 14	**16**	*25* 20 15 *14* 19	**19**
	24	*4* 6 *12* *12* 8	**8**	10 13 *15* 10 *8*	**11**	*22* *16* 18 20 19	**19**	*28* 22 23 22 *21*	**23**
	30	*7* *7* *12* 11 11	**10**	*12* 16 17 14 *18*	***15**	*19* *25* 24 23 21	***22**	*39* 27 28 *23* 31	***30**
	35	14 *10* *16* 14 *16*	***14**	15 *18* 17 *12* 13	***15**	*20* 17 16 16 *15*	**17**	18 18 18 *16* *22*	**18**
	40	*10* 12 *15* 12 13	**12**	*16* *16* 12 *10* 12	**13**	*13* *13* *18* 14 16	**15**	*22* *11* 16 14 17	**16**
	45	*8* 10 *8* *11* 10	**9**	8 *7* *12* 11 9	**9**	10 *5* *12* 10 6	**9**	8 *13* *6* 11 7	**9**
Cultures with air flow of 1 liter per day	12	4 4 *5* *3* 4	**4**	*12* *7* 8 *7* *12*	**9**	11 *14* 12 *7* 10	**11**	14 15 *19* *12* 13	**15**
	19	*4* 5 5 *4* *8*	**5**	9 11 12 *8* *13*	**11**	24 *18* *25* *18* 21	**21**	*67* 48 57 *35* 51	**52**
	24	*12* *5* 9 *13* *13*	**10**	*15* 18 16 *23* 22	**19**	63 62 56 *73* *53*	***61**	85 86 85 *87* *84*	***85**
	30	11 11 *10* *15* *10*	**11**	35 *42* *29* 25 37	***33**	45 44 *36* 48 *51*	**45**	*65* *48* 54 51 60	**56**
	35	13 12 *16* 12 *10*	***13**	19 *21* *11* 17 17	**17**	20 18 22 *23* *14*	**19**	*19* 21 *26* 22 23	**22**
	40	*10* 14 11 11 *15*	**12**	11 11 *9* *14* 13	**12**	14 11 16 *17* 9	**15**	18 *11* *11* *21* 20	**16**
	45	7 *3* *11* *11* 7	**8**	8 8 8 *7* *10*	**8**	*7* 9 *7* 8 *11*	**8**	8 8 *7* *9* 8	**8**
Cultures with air flow of 3 liters per day	12	*5* *3* *3* *5* *5*	**4**	*12* 7 9 9 *5*	**8**	*9* 10 *15* 12 14	**12**	18 *14* *23* 21 19	**19**
	19	10 8 *11* *7* 9	**9**	*15* 11 11 *9* 11	**11**	21 24 *26* *20* 21	**22**	52 58 *71* 52 *46*	**56**
	24	*13* *9* *13* 10 11	**11**	21 *23* *18* 22 20	**21**	*58* *33* 45 46 46	**46**	63 *61* 74 *77* 70	***69**
	30	*12* *19* 17 14 16	**16**	*43* 39 32 *31* 42	***37**	45 *40* *67* 53 55	***52**	*57* *71* 69 67 69	**67**
	35	18 *20* *14* 17 15	***17**	*21* 25 25 *28* 22	**24**	28 *20* *29* 28 *29*	**27**	*23* 26 27 *34* *23*	**27**
	40	16 *14* 16 *22* 16	***17**	*11* 16 21 *26* 16	**18**	18 15 18 *27* *13*	**18**	16 *15* 18 *25* *15*	**18**
	45	13 13 *11* *14* *14*	13	15 13 12 *9* *17*	**13**	*16* *10* 14 13 *10*	**13**	*18* *9* 12 11 16	**13**

* Maximum average.

TABLE I (*continued*)

	Temperature	For 6-hour period		For 12-hour period		For 18-hour period		For 24-hour period	
		Percentages for 5 tests	Average	Percentages for 5 tests	Average	Percentages for 5 tests	Average	Percentages for 5 tests	Average
Cultures	*deg. C.*		*per cent.*		*per cent.*		*per cent.*		*per cent.*
with air	12	5 *3* *6* *3* *3*	4	*9* *7* *9* 7 8	8	19 *21* *13* 15 19	17	27 26 *30* *15* 25	25
flow of	19	*5* 10 7 *16* 8	9	19 20 *23* *16* *16*	20	*27* *45* 39 28 *27*	33	68 74 66 *65* *77*	70
6 liters	24	*8* *15* *15* *8* *8*	11	*45* *38* 39 39 *38*	40	*83* 77 78 *75* 79	*78	*90* *90* 94 91 *95*	*92
per day	30	*21* *10* 11 12 18	14	63 *49* 53 53 *66*	*58	*67* *67* 65 65 *60*	65	*80* 73 79 79 *65*	75
	35	18 21 17 *24* *15*	*19	*26* 28 29 27 *30*	28	27 27 *33* 28 *24*	28	*32* *28* *32* 29 31	30
	40	*24* 15 22 19 *10*	18	21 *26* 23 *18* 21	22	21 24 *25* *19* *19*	22	20 *29* *17* 19 27	22
	45	*27* *8* 12 14 11	14	*19* 16 13 12 *11*	14	*16* *10* 12 15 *16*	14	13 *21* 14 14 *13*	15
Cultures	12	*3* *3* *7* *3* 4	4	*11* 10 *7* 9 *7*	9	*10* 13 *18* 17 12	14	30 *36* 29 *22* 29	29
with air	19	9 8 *10* 6 *5*	8	12 22 *23* 19 *10*	17	*29* 38 50 *55* 50	44	*90* 80 *62* 71 76	76
flow of	24	17 15 *19* 13 *10*	15	*44* *42* 43 43 43	43	81 *70* 83 *95* 85	*83	*90* *99* 96 93 *99*	*95
15 liters	30	*22* 20 *16* 20 *16*	19	*68* 54 67 56 *53*	*60	*90* 85 85 *75* 79	*83	*90* 84 88 80 *79*	84
per day	35	20 *23* *19* 21 *19*	*20	*35* *22* 31 28 32	30	*43* 30 *25* 27 31	31	36 *38* 31 *30* 31	33
	40	*26* 20 21 *17* *17*	*20	25 24 *28* *15* 20	22	*19* 22 22 *26* 22	22	*17* 20 20 22 *32*	22
	45	*5* 15 *19* 18 17	15	*9* 19 *20* 14 12	15	16 15 *24* *11* *11*	15	*10* 11 14 *20* 18	15
Cultures	12	*4* 3 3 *2* 3	3	*6* 10 *19* 8 16	12	16 *22* *12* 17 *12*	16	31 *35* *35* *22* 23	29
with air	19	*9* 6 *9* *9* *4*	7	22 *19* *27* 22 24	23	45 *66* 57 *39* 46	51	*94* 92 86 71 *65*	82
flow of	24	*12* *7* 8 *12* 11	10	57 *59* 41 *30* 42	46	*97* 92 91 81 59	84	99 *100* 95 *91* 89	*95
30 liters	30	19 17 17 *20* *12*	17	70 *81* 78 *64* 75	*74	90 88 *91* 80 *79*	*86	*99* 94 95 93 *87*	93
per day	35	17 *13* 21 19 *24*	*19	29 *34* *19* 32 28	28	*44* *22* 33 *44* 35	36	53 35 *34* 49 *55*	45
	40	20 22 15 *12* *24*	*19	*23* *23* 20 20 *14*	20	20 21 *23* 20 *18*	20	*27* 22 21 19 *15*	21
	45	*11* *18* 15 17 16	15	14 14 17 *18* *12*	15	13 16 *17* *12* *17*	15	*11* *19* 16 13 14	15

* Maximum average.

periods used and the six horizontal sections represent the different aeration treatments. Each group of 35 values thus represents a single experiment performed five times. For each time an experiment was performed there are 7 percentage values, corresponding to the seven different maintained temperatures. The minimum and the maximum in each horizontal series of five values from like tests are in italics and the average of each five corresponding values is shown in boldface type. There are 168 of these averages. The highest average for each experiment is marked by an asterisk. Of course the temperature corresponding to this highest average represents about the optimal temperature for seedling production in the experiment in question. For some experiments there are two highest averages, of the same magnitude, which of course implies that the optimal temperature for that experiment may be considered as lying somewhere between the two temperatures thus indicated, or else slightly below the lower one or slightly above the higher one.

It may be noted that most low percentage values may be considered as relatively less precise than high ones. To be counted as germinated at the end of any test a seed must have burst its seedcoat, though some additional growth may have occurred. At the end of an 18-hr. or 24-hr. period with good conditions for germination many seedlings had elongated considerably, *i.e.*, their seeds had passed the critical moment of bursting the seedcoats. But no account is taken in table I of this over-growth, since whenever over-growth occurred there are data for one or more shorter periods. Thus, for example, the average percentage value of 67 for 30°, 3 l. and 24 hr. obviously includes the seeds that had germinated in the 18-hr., 12-hr. and 6-hr. periods. But the value (**16**) for the 6-hr. period under these conditions represents no considerable over-growth. Such considerations are clearly cared for if 6-hr. increments of the average percentages are computed by subtraction and that mode of presentation will be employed below (table II).

On the other hand, at the end of any period a seed might not yet have burst its seedcoat, being consequently counted as ungerminated, but the processes leading to germination might have progressed very far; such a seed might have been recorded as germinated if the period had been an hour or two longer. As the percentage value is higher there is less probability of nearly-germinated seeds, since the maximum possible value in any case can not be above 100. In any case this lack of precision can never amount to more than a few units of the average percentage value. Furthermore, it is not to be considered at all in instances where the next longer period showed no increase; for example, the value 27, for 35°, 3 l. and 18 hr. can not lack precision in this respect because the corresponding 24-hr. period shows exactly the same average percentage as is shown by this 18-hr. period.

It is at once apparent from table I that, within the ranges dealt with, all three experimental variables (maintained temperature, aeration treatment and duration of incubation) were influential in determining the percentages and their averages. The *lowest germination percentages* correspond to environmental complexes that had either low or high temperatures, low degrees of aeration or short periods, or two of these features or all three of them together. The actual minimum percentage is 1 (for the third test of 12° without air flow and with a 6-hr. incubation period) and the lowest average percentage is 3 (for 12°, 6 hr. and air flow of 30 l.). Averages below 5 represent all the 6-hr. tests with 12° and all other tests have averages of 5 or above. Averages of 10 or below may be taken as representing the least efficient environmental complexes studied. These are confined to the following combinations: All 6-hr. tests with 12° and all 12-hr. tests with that temperature, excepting the one with air flow of 30 l. (which gave an average of only 12); all 6-hr. tests with 19°; all tests with 24° and without air flow or with air flow of 1 l. or 30 l.; and all 6-hr., 12-hr., 18-hr. and 24-hr. tests with 45° and without air flow or with air flow of 1 l.

The *highest germination percentages* correspond to combinations of 24° or 30° with 18-hr. or 24-hr. periods and air flow of 6 l., 15 l., or 30 l. Average values above 90 are confined to the 24-hr. tests with 24° and air flow of 6 l., 15 l., or 30 l., and with 30° and air flow of 30 l.

The temperature graphs

Many details concerning the relations of the various environmental combinations included in this study are most readily brought out by means of graphs. The average percentages shown in boldface type in table I may be plotted in many ways, only a few of which will be considered in detail here. Attention will be confined to the graphs on which the percentages are plotted as ordinates. These are of three sorts: (A) those on which temperature values are abscissas (24 graphs), (B) those on which aeration indices are abscissas (28 graphs) and (C) those on which the time indices are abscissas (42 graphs). Each of these three groups of graphs may be arranged for convenient study in two ways. For the first group the 24 temperature graphs may be arranged (A 1) in 6 sheaves of 4 graphs each, every sheaf representing a single aeration treatment while each individual graph represents a single length of incubation period; or they may be arranged (A 2) in 4 sheaves (for the incubation periods) of 6 graphs each (for the aeration treatments). The 28 aeration graphs may be assembled (B 1) in 7 sheaves (for the temperatures) of 4 graphs each (for the times of incubation), or (B 2) in 4 sheaves (times of incubation) of 7 graphs each (temperatures). In like manner the 42 duration graphs may be assembled (C 1) in 7 sheaves (temperatures) of 6 graphs each (aeration treat-

ments) or (C 2) in 6 sheaves (aeration treatments) of 7 graphs each (temperatures). All of these sets of graphs may be readily constructed from the data given in table I. A few of them are presented here, in figures 1–4.

Figure 1 gives representative samples of arrangement A 1. Three of the six sheaves are shown, for cultures without air flow and with air flow of

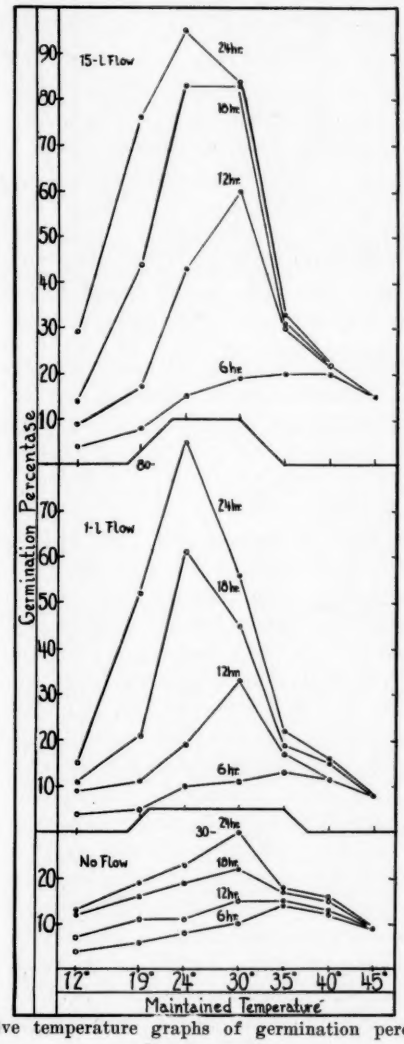

FIG. 1. Representative temperature graphs of germination percentage, grouped according to aeration.

1 l. and 15 l. per day. Each sheaf comprises four individual graphs, one for each of the four incubation periods.

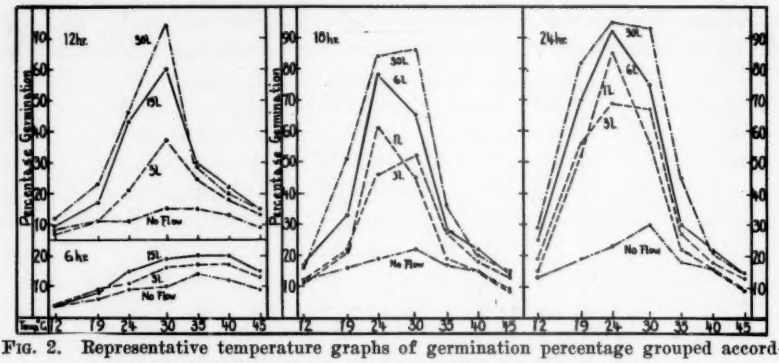

FIG. 2. Representative temperature graphs of germination percentage grouped according to length of incubation period. ("Percentage germination" should be *germination percentage.*)

Figure 2 presents arrangement A 2, with four sheaves, one for each incubation period. For the sake of clearness one or more graphs are omitted from each sheaf but the general relations are adequately shown.

Some features brought out by these temperature graphs will now be mentioned, reference being made to table I and figures 1 and 2. It is seen that the form of the temperature graph differs markedly according to period length and aeration treatment. All of the 24 graphs agree, as would be expected, in showing relatively low percentage values for 12° and for 45°, with relatively higher values for the intervening temperatures. No minimal nor maximal temperature for germination is shown but the percentages for all tests with 6-hr. period and a temperature of 12° closely approach zero, which suggests that the minimal temperature for germination in 6 hours must have been not far below 12°. For the longer incubation periods the percentages for 12° are progressively higher (for the 24-hr. period, more divergent) and it is suggested that the corresponding minimal temperatures may have been increasingly below 12° with progressively more vigorous aerations as well as with progressively longer periods. The temperature minimum for seedling production by this lot of wheat seed may consequently be said to have been more or less below 12° for all tests, probably farther below that temperature for the tests with longer periods, especially with the more vigorous aeration treatments. For ready comparison, all the average percentages for 12° are shown on page 227.

For the few seeds that germinated at 12° in 6 hr. the aeration differences were apparently without influence, but for those that germinated in longer

	For 6-hr. period, 12°	For 12-hr. period, 12°	For 18-hr. period, 12°	For 24-hr. period, 12°
No flow	4	7	12	13
1-l. flow	4	9	11	15
3-l. flow	4	8	12	19
6-l. flow	4	8	17	25
15-l. flow	4	9	14	29
30-l. flow	3	12	16	29

periods the more vigorous aeration treatments gave appreciably higher percentages. For each aeration treatment the longer the period of incubation the higher is the germination percentage, as would be expected. But the highest germination percentage obtained for 12° was only 29 (for 24 hr. and air flow of 15 l. and 30 l.). It is apparent that no considerably higher percentages than those given would have been obtained for a temperature of 12° combined with any one of these four incubation periods if the air flow had been still more rapid. But it is highly probable that any 24-hr. value for 12° would have been notably surpassed with any one of these aeration treatments if the incubation period had been sufficiently longer than 24-hr.

Turning to the germination percentages shown for the highest temperature employed (45°), all four periods gave the same percentage of germination with all aeration treatments, which indicates that no additional seeds germinated at 45° after the first 6 hr. of incubation, no matter what aeration treatment was employed. It is not likely that a period longer than 24 hr. would have given any additional germination with this temperature for any aeration treatment in the range of treatments studied. This temperature was surely supra-optimal and consequently probably increasingly injurious as time elapsed; those seeds that could germinate quickly under these conditions might do so but those that were delayed more than 6 hr. were unable to germinate at all. This thought is the same as was suggested by Haasis (7) for a similar state of affairs with his conifer seeds. Although differences in period length did not influence the germination percentage for 45° with any aeration treatment tested, as has just been noted, it is remarkable that the percentage value for this highest temperature combined with any length of period is higher for air flows of 3 l., 6 l., 15 l. or 30 l. than for no flow or for a flow of only 1 l. per day. All the percentage values for 45° are shown on the following page, for comparison among themselves and with those for 12°, given above.

It is indicated that the maximal temperature for germination of this lot of seed was always considerably above 45°, apparently somewhat farther above that temperature with air flow of 3 l. or more but apparently not

	For 6-hr. period, 45°	For 12-hr. period, 45°	For 18-hr. period, 45°	For 24-hr. period, 45°
No flow	9	9	9	9
1-l. flow	8	8	8	8
3-l. flow	13	13	13	13
6-l. flow	14	14	14	15
15-l. flow	15	15	15	15
30-l. flow	15	15	15	15

any higher, for any aeration treatment, with an incubation period of 12, 18 or 24 hr. than with a period of only 6 hr.

With regard to the intermediate temperatures tested (19°, 24°, 30°, 35°, and 40°), all the temperature graphs have the usual form, with a generally well defined graph maximum, the abscissa of which is to be taken as about the temperature optimum in each instance. For any aeration treatment this maximum is always progressively greater with longer periods (fig. 1), as might be expected, and it is generally greater for more vigorous than for less vigorous aeration (fig. 2). The lowest value of this graph maximum is 13 or 14 per cent. (for 6 hr. and 35°, with no flow or a flow of 1 l. per day) and its highest value is 92 or 95 per cent. (for 24 hr. and 24°, with air flow of 15 l. or 30 l. per day). The values for all temperature-graph maxima (maximal ordinates) are shown below, the corresponding abscissa value (about the optimal temperature) being shown in parenthesis in each case.

	Graph maximum for 6-hr. period	Graph maximum for 12-hr. period	Graph maximum for 18-hr. period	Graph maximum for 24-hr. period
No flow	14 (35°)	15 (30°, 35°)	22 (30°)	30 (30°)
1-l. flow	13 (35°)	33 (30°)	*61 (24°)	*85 (24°)
3-l. flow	17 (30°, 35°)	37 (30°)	*52 (30°)	*69 (24°)
6-l. flow	19 (30°)	58 (30°)	78 (24°)	92 (24°)
15-l. flow	20 (35°, 40°)	60 (30°)	83 (24°, 30°)	95 (24°)
30-l. flow	19 (35°, 40°)	74 (30°)	86 (30°)	95 (24°)

As to the general relation of aeration treatment to the maximal percentage value for any length of period, there are four exceptions to the statement that the maximum is greater for more vigorous than for less vigorous aeration: (1) the 6-hr. maximum for a flow of 1 l. (13 per cent.) is not greater than that for no air flow (14 per cent.); (2) the 6-hr. maximum for a 30-l. flow (19 per cent.) is not greater than that for a 15-l. flow (20 per cent.); (3) the 18-hr. maximum for a 3-l. flow (52 per cent.) is

clearly smaller than that for a 1-l. flow (61 per cent.); and (4) the 24-hr. maximum for a 3-l. flow (69 per cent.) is much smaller than that for a 1-l. flow (85 per cent.). The first two of these exceptions may be regarded as not significant but the last two appear to be worthy of careful consideration and their values are marked with asterisks in the above tabulation. They will be reverted to later.

One of the most striking features of the temperature relations here in question is the regression of the optimal temperature with increasing length of the incubation period, for any aeration treatment (fig. 1). It is not quite regular, but there appears to be no doubt of its significance. Other conditions being nearly alike, the optimal temperature for the production of seedlings by this lot of seed is generally lower for longer incubation. If the whole range of period lengths dealt with is considered there are no exceptions to this and the total regression is from about 30° or 35° (or even higher) to about 24°.

A similar regression of the optimal temperature for germination was reported by HAASIS for his conifer seeds. In some cases, as has been said, he found also a double temperature optimum when the length of the incubation period was properly chosen, other influences being the same and favorable to germination, but no indication of a double temperature optimum (bimodal temperature curve) was observed in the present study.

A change in temperature optimum with lapse of time is apparently generally characteristic of growth rates, as has been pointed out by LEHENBAUER (**11**), TALMA (quoted by BENECKE-JOST, **2**, p. 37), FAWCETT (**5**) and GERICKE (**6**); but it does not appear to have been emphasized for germination percentage (which is of course fundamentally different from growth rate) excepting by HAASIS. The general question of growth retardation with the lapse of time, when temperature and other influences are maintained, was discussed with characteristic acumen by F. F. BLACKMAN (**3**).

TEMPERATURE OPTIMA BASED ON AVERAGE HOURLY RATES OF SEEDLING PRODUCTION

To compute the average hourly rate of seedling production corresponding to the highest percentage value for each combination of aeration treatment and duration of incubation we divide each of the maximal percentages by the length of the period that gave it. For example, 6 hr. of a 6-l. air flow gave a maximal germination percentage of 19 (30°) and an average rate of 3.2 seedlings per hour; 12 hr. of a 30-l. flow gave a maximal percentage of 74 (30°) and an average rate of 6.2 seedlings per hour; etc. The average hourly rate of maximal seedling production for all the combinations of aeration treatment and duration are shown on page 230, with the corresponding temperatures in parenthesis.

	Highest average hourly rates for 6-hr. period	Highest average hourly rates for 12-hr. period	Highest average hourly rates for 18-hr. period	Highest average hourly rates for 24-hr. period
No flow	2.3 (35°)	1.3 (30°, 35°)	1.2 (30°)	1.3 (30°)
1-l. flow	2.2 (35°)	2.8 (30°)	3.4 (24°)	3.5 (24°)
3-l. flow	2.8 (30°, 35°)	3.1 (30°)	2.9 (30°)	2.9 (24°)
6-l. flow	3.2 (30°)	4.8 (30°)	4.3 (24°)	3.8 (24°)
15-l. flow	3.3 (35°, 40°)	5.0 (30°)	4.6 (24°, 30°)	4.0 (24°)
30-l. flow	3.2 (35°, 40°)	6.2 (30°)	4.8 (30°)	4.0 (24°)

It is seen that this highest average hourly rate was, for each period length, significantly greater with air flow of 6 l., 15 l. or 30 l. than with less vigorous aeration treatments and that for all period lengths excepting the shortest one it was significantly greater with air flow of 1 l. or 3 l. than without any air flow. Furthermore, with air flows of 6 l., 15 l. and 30 l., this index of efficiency has its greatest magnitude (4.8, 5.0, 6.2) for the 12-hr. period and its least magnitude (3.2, 3.3, 3.2) for the 6-hr. period; but this magnitude is about the same (2.8, 3.1, 2.9, 2.9) for all four periods with a flow of 3 l., while it is greatest for the 18-hr. and 24-hr. periods (3.4, 3.5) with a flow of 1 l. Without air flow it is greatest (2.3) for the shortest period.

With regard to average hourly rates the optimal combination of temperature, aeration treatment and time is shown as that for 30°, 30-l. flow and 12 hr., which environmental combination gave the hourly rate of 6.2. The next to the highest rate is 5.0 (for 30°, 15-l. flow, 12 hr.) and a rate of 4.8 is shown alike for the combination 30°, 6-l. flow, 12 hr., and for the combination 30°, 30-l. flow and 12 hr. The highest hourly rate shown for 24° in any combination is very much lower than the ones for 30°; it is 4.6 for the combination of 24°, 15-l. flow, 18 hr., but this same rate is given for the combination 30°, 15-l. flow, 18 hr. and the highest 24°-rate not equalled or surpassed by some 30°-rate is only 4.3, for the combination 24°, 6-l. flow, 18 hr. The optimal temperature for average hourly production of seedlings is clearly 30° and not 24°.

The aeration graphs

Figure 3 presents aeration graphs of germination percentage for the 6-hr. and the 24-hr. incubation periods. They are representative of the group of 42 graphs previously referred to as B 2. The graphs for 12°, 19°, 24°, and 30° are continuous lines and those for 35°, 40°, and 45° are broken lines. To save space, two sections of this figure have been cut out, as is indicated, but all points of observation are shown.

The percentage values for the 6-hr. period are all low and about all that can be said of them in general is that more vigorous aeration treatment for

any temperature usually shows somewhat higher percentages, up to an air flow of 6 l. per day, while still more rapid rates of air flow show no significantly greater percentages. No influence of aeration treatment is evident for the 6-hr. period and 12°. For the 6-hr. period and 24° an optimal air flow appears as 15 l. per day and it is suggested that the 30-l. flow may have been supraoptimal for 30° and 35° as well as for 24°. For the 12-hr.

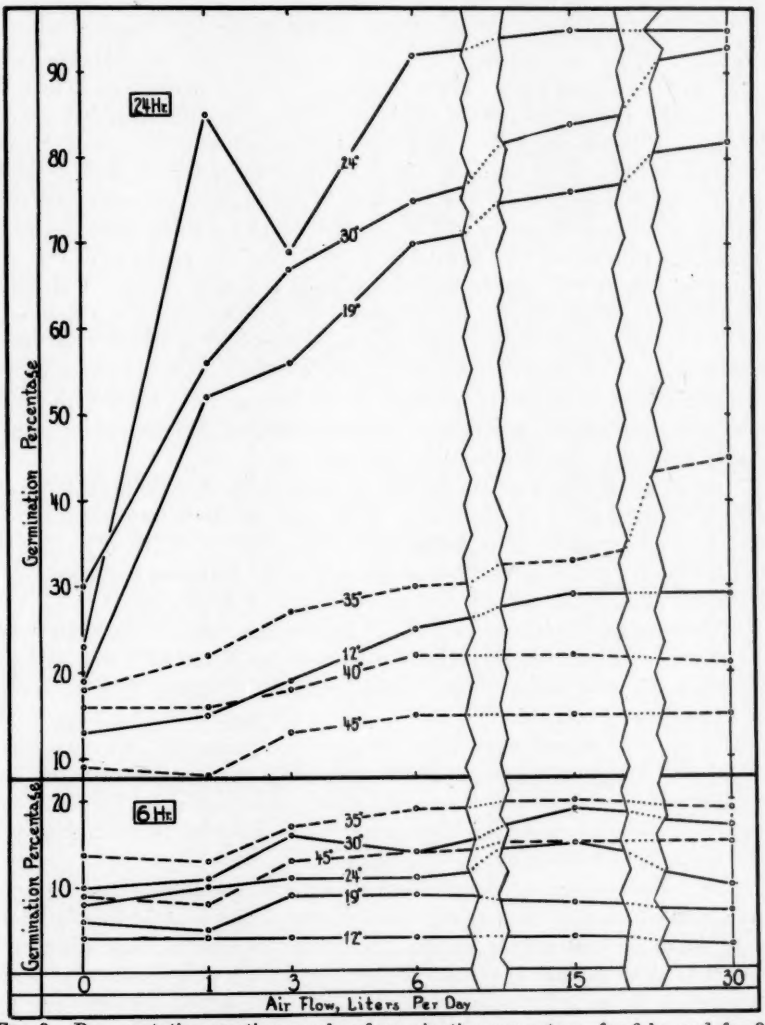

FIG. 3. Representative aeration graphs of germination percentage, for 6 hr. and for 24 hr. of incubation. Two portions are cut out to save space.

period (for which no graphs are shown) no optimal aeration treatment appears for 24° or 30°, and a flow of 30 l. per day gave percentages for the other temperatures just as high as were given for a flow of 6 l. For the 18-hr. period (graphs not shown) the temperatures for which no aeration optimum was attained are 19° and 35°; the 6-l. flow gave as high values as the 30-l. flow for 40° and 45° and the 15-l. flow gave as high values as the 30-l. flow for 24° and 30°.

The sheaf of graphs for the 24-hr. period (figure 3) deserves special attention. They are not very different from those for the 18-hr. period, which are not shown here. The average germination percentage is seen to be generally higher with more vigorous aeration, but for temperatures of 40° and 45° this is not true for air flows more rapid than 6 l. per day, and for temperatures of 12° and 24° the percentage value for the 30-l. air flow is not greater than that for the 15-l. flow. The most consistently upward-sloping graphs are those for 19°, 30° and 35° and for these three temperatures some rate of air flow greater than 30 l. per day might have given a percentage value still higher than the highest one here shown. This suggests that, for these three temperatures, the optimal rate of air flow was not attained, though it appears to have been attained for 12°, 24°, 40° and 45°, within the ranges of conditions here studied. The general air-flow optimum is seen to be the range from 6 l. to 30 l. and any more rapid flow could not have given a percentage much above 93 or 95, since these values closely approach the fixed limit of 100 per cent.

The 24-hr. aeration graph for 24° is remarkable for an apparent secondary minimal point for the 3-l. air flow, which has been mentioned above. This kind of irregularity is definitely shown only for 24 hr., 3 l. (fig. 3) and for 18 hr., 24°, 3 l., but it is suggested by the forms of the graphs for 24 hr., 19°, 3 l., for 18 hr., 19°, 3 l. and perhaps for 12 hr., 30°, 3 l.

This secondary minimal point is of special interest in connection with the double oxygen optima recently reported by MACK (17) for carbon-dioxide production and shoot elongation in wheat seedlings from the same lot of seed as was used in this study. In the present case it appears that, with a properly chosen maintained temperature (about 24°, which is near the general temperature optimum for this lot of seed) an air flow of 3 l. per day gave a markedly lower germination percentage in 18 hr. or 24 hr. than was given by either less vigorous or more vigorous aeration with the same periods of incubation.

These observations on aeration optima may be important when we shall have learned more of the oxygen requirements of germinating seeds. For many kinds of seed oxygen supply appears to be just as truly important and critical as temperature and water supply are, and aeration of such cultures as those of the present study may be effective—as has been re-

marked—in other ways than through its influence on oxygen supply. To secure very high germination percentages (approaching 100) in 24 hr. or 18 hr., with this lot of seed under dilute nutrient solution, it was only necessary to find the correct combination of temperature, aeration treatment and length of incubation period.

THREE-DIMENSION GRAPHS

Another way to bring the average percentages of table I together for somewhat ready comparison, and to show how markedly different environmental complexes gave similar results, is to plot the percentages on three-dimensional diagrams, with contour lines or isopleths connecting points of like value. Since there are four variables to be dealt with and only three may be plotted on a single diagram, several diagrams are requisite to represent all the relations. If we consider the diagrams as representing curved surfaces and always plot the average percentages as altitudes, three sets of diagrams are possible. These may be characterized as follows, the terminology being that of a topographic chart.

SET A.—West-east distances represent the different degrees of aeration treatment and south-north distances represent temperature. There are four diagrams in this set, one for each incubation period. The set for 24 hr. is shown in figure 4 (A).

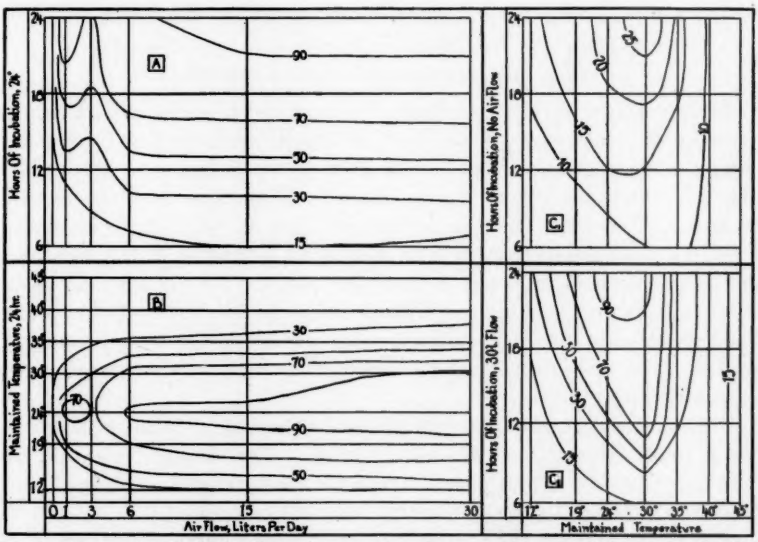

FIG. 4 Representative three-dimension graphs of germination percentage as related to maintained temperature, aeration treatment and duration of incubation.

SET B.—West-east distances represent aeration treatments, as in set A, but south-north distances represent different incubation periods. In this set there are seven diagrams, one for each temperature. The set for 24° is shown in figure 4 (B).

SET. C.—West-east distances represent temperature and south-north distances represent, as in set B, the different incubation periods. There are six diagrams, one for each aeration treatment. The sets for no air flow and for a flow of 30 l. per day are shown in figure 4 (C_1 and C_2).

Each isopleth or contour line of each diagram passes through points of like altitude and all points on any line consequently represent conditional complexes giving about the same average germination percentage. Thus each diagram is seen to be divided into areas by the contours, like a physiographic chart of a terrain. The percentage values are shown on the contours. Each diagram has a "high" and a "low" area and several intermediate ones.

These isopleth diagrams bring out the relations already mentioned and many others in addition, but they need not be discussed in detail. This method of presentation of the numerical values shows that the same percentage value was given by many different treatments of the cultures and that different but equally effective treatments are consistently related to the system of coordinates.

SIX-HOUR AND TWELVE-HOUR INCREMENTS OF THE AVERAGE PERCENTAGE VALUES FOR THE TWENTY-FOUR HOURS AFTER THE BEGINNING OF THE EXPERIMENTS

If we consider that the number of germinated seeds in any culture increased with time we may derive 6-hr. and 12-hr. increments by the ordinary method of subtraction. The results of these operations are set forth in table II, which consists of six horizontal sections, one for each aeration treatment. In each section are seven lines, one for each of the seven temperatures, and each line presents, in order from left to right, the increments for the first, second, third and fourth 6-hr. periods, for the first and second 12-hr. periods and the total percentage value for the entire 24-hr. period. The highest value for each period in each section of the table is shown in boldface type and the highest 6-hr. value for each aeration treatment is designated by an asterisk.

The temperature giving the greatest 6-hr. increment value with any air flow is progressively lower for later periods. For example, with air flow of 15 l. per day the optimal temperature for seedling production in the first 6 hours is shown as 35° or 40°, for the second 6-hr. period it is 30°, for the third it is 24° and for the fourth it is 19°. This relation holds for all air flows tested but does not appear to hold for the cultures without air flow.

TABLE II

SIX-HOUR AND TWELVE-HOUR INCREMENTS OF THE AVERAGE PERCENTAGES SHOWN IN TABLE I

AERATION	TEMPERATURE	FOR 1ST 6 HRS.	FOR 2ND 6 HRS.	FOR 3RD 6 HRS.	FOR 4TH 6 HRS.	FOR 1ST. 12 HRS.	FOR 2ND 12 HRS.	FOR 1ST 24 HRS.
Cultures without air flow	*deg. C.*							
	12	4	3	5	1	7	6	13
	19	6	**5**	5	3	11	**8**	**19**
	24	8	3	**8**	4	11	12	**23**
	30	10	**5**	7	**8**	**15**	**15**	**30**
	35	***14**	1	2	1	**15**	3	18
	40	12	1	2	1	13	3	16
	45	9	0	0	0	9	0	9
Cultures with air flow of 1 l. per day	12	4	5	2	4	9	6	15
	19	5	6	10	**31**	11	41	52
	24	10	9	***42**	24	19	**66**	**85**
	30	11	**22**	12	11	**33**	23	56
	35	**13**	4	2	3	17	5	22
	40	12	0	3	1	12	4	16
	45	8	0	0	0	8	0	8
Cultures with air flow of 3 l. per day	12	4	4	4	7	8	11	19
	19	9	2	11	***34**	11	45	56
	24	11	10	**25**	23	21	**48**	**69**
	30	16	**21**	15	15	**37**	30	67
	35	**17**	7	3	0	24	3	27
	40	**17**	1	0	0	18	0	18
	45	13	0	0	0	13	0	13

* Highest 6-hr. value for the given aeration treatment.

TABLE II (*continued*)

Aeration	Temperature	For 1st 6 hrs.	For 2nd 6 hrs.	For 3rd 6 hrs.	For 4th 6 hrs.	For 1st 12 hrs.	For 2nd 12 hrs.	For 1st 24 hrs.
Cultures	*deg. C.*							
with	12	4	4	9	8	8	17	25
air flow	19	9	11	13	**37**	20	50	70
of 6 l.	24	11	29	**38**	14	40	**52**	**92**
per day	30	14	*44	7	10	**58**	17	75
	35	**19**	9	0	2	28	2	30
	40	18	4	0	0	22	0	22
	45	14	0	0	1	14	1	15
Cultures	12	4	5	5	15	9	20	29
with	19	8	9	27	**32**	17	**59**	76
air flow	24	15	28	**40**	12	43	52	**95**
of 15 l.	30	19	*41	23	1	**60**	24	84
per day	35	**20**	10	1	2	30	3	33
	40	**20**	2	0	0	22	0	22
	45	15	0	0	0	15	0	15
Cultures	12	3	9	4	13	12	17	29
with	19	7	16	28	**31**	23	**59**	82
air flow	24	10	36	**38**	11	46	49	**95**
of 30 l.	30	17	*57	12	7	**74**	19	93
per day	35	**19**	9	8	9	28	17	45
	40	**19**	1	0	1	20	1	21
	45	15	0	0	0	15	0	15

* Highest 6-hr. value for the given aeration treatment.

Of course this generalization constitutes a step in the analysis of the reasons for the temperature regression already noted.

If we arrange the 6-hr.-increment values that are above 30 per cent. in the descending order of their magnitudes we obtain the following list. Opposite each increment value is shown the particular combination of temperature and air flow that gave it and the number of the 6-hr. period for which it occurs. With the single exception of the value 36, every one of

6-HR. INCREMENT	TEMPERATURE	RATE OF AIR FLOW	NO. OF PERIOD
per cent.	*deg. C.*	*l. per day*	
57	30	30	2
44	30	6	2
42	24	1	3
41	30	15	2
40	24	15	3
38	24	30.6	3
37	19	6	4
36	24	34	2
34	19	3	4
32	19	15	4
31	19	1,30	4

these corresponds to one or another of the three temperature-period combinations, 30°, 2nd period; 24°, 3rd period; and 19°, 4th period. The aeration relations of these best combinations are shown by the upper three graphs of figure 5. In that figure abscissas are aeration treatments and ordinates are 6-hr. germination-percentage increments. Ordinate values are shown by the numerals on the graphs. The graph for 35°, 1st period, is added for comparison.

The three upper graphs illustrate how great may be the difference between cultures without artificial aeration and those with an air flow of 1 l. per day. The cultures without air flow had practically no convection and the oxygen supply to the seeds must have been very slow indeed. Perhaps the marked effect of the slowest rate of bubbling, in giving increment values higher than the corresponding ones without air flow, may have been due to stirring of the solution rather than to the entrance of air into the flasks. A double optimum is again shown on the graph for 24°, 3rd period, and it is suggested for 30°, 2nd period, the increment value for a 1-l. flow being higher than that for a 3-l. flow. For air flows of from 6 l. to 30 l. per day the 6-hr. increment values are about the same on each one of the lower three graphs, but the second 6-hr. period with 30° gave the highest increment (57 per cent.) with the most rapid air flow and might have given still higher values with still more rapid rates of flow.

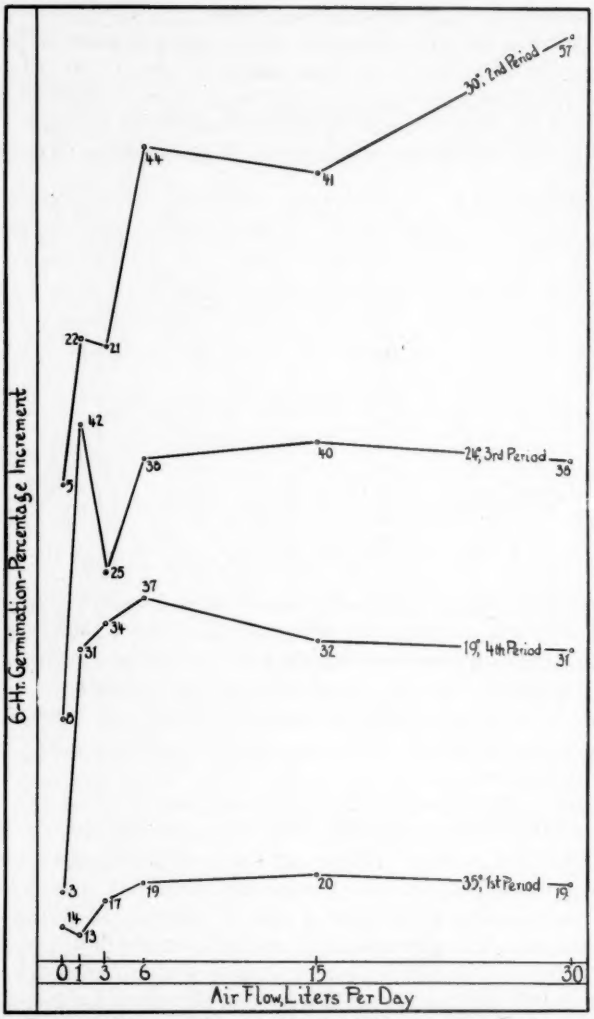

FIG. 5. Representative aeration graphs of the 6-hr. increments of germination percentage. The graphs are arranged to avoid intersections, without reference to the scale of ordinates, but all are plotted on the same scale, ordinate values being shown at the points of record.

If it were desired to select a large number of seedlings as nearly alike as possible, as for a series of solution cultures, about 50 per cent. of this lot of seed might be so selected by taking just those seedlings that had burst the seedcoats in the second 6 hours of incubation under the weak nutrient solution here used and with maintained temperature of 30° and air flow of 30 l. per day. This is an illustration of the manner in which a number of physiologically similar seedlings may be selected by means of differential culture, as has been pointed out by HAASIS. Of course this sort of selection cannot be applied without bringing the seeds into activity and the seeds selected cannot be returned to ordinary storage; they must be allowed to continue their development after it is once started, unless, indeed, they might be held in a kind of dormancy by the employment of suitably low temperature. A study of table II will suggest many ways by which several groups or categories of seedlings might be secured, the individuals of each group being nearly alike by the physiological test of germination in the same short period under a standard set of environmental conditions, while the several groups would differ among themselves in more or less pronounced ways. Seedlings produced in the first 6 hours of incubation with a specified environmental complex might be very different in physiological characteristics from those produced in the fourth 6-hr. period under the same conditions, for example. Whether such differences might be related to genetic characteristics is a question that may be interesting and important.

It will be noted from table II that, for all aeration treatments, germination at 45° was confined to the first 6-hr. period, which has already been mentioned, and that the same is practically true for 40°. With a temperature of 35° most of the germination that occurred is shown for the first 6-hr. period in every case, but the second period gave considerable germination with air flows more rapid than 1 l. per day. With this same temperature still later periods show increments that are nearly or quite negligible, excepting in the case of the most vigorous aeration. These three temperatures (45°, 40°, and 35°) are all above the general temperature optimum for this lot of seed and it appears that they agreed in giving the highest germination percentage for the first 6 hr.

Besides the relations here mentioned, other interesting relations may be noted by a study of table II. The 12-hr. increments of average germination percentage show about the same relations, *mutatis mutandis*, as do the 6-hr. increments and they need not be dwelt on here, for the 6-hr. increments are more useful in studying these relations.

OPTIMAL CONDITIONAL COMPLEXES

From what has been said in the preceding sections it is clear that there are at least three quite different criteria by which we may judge which ones

of the many different combinations of temperature, aeration treatment and incubation period were optimal.

(1) In the first place, most students of germination percentage or seed viability would probably regard as optimal, within the ranges of this study, those sets of conditions that gave the highest average percentages, without regard to time. On that basis, as has been pointed out, we may name three combinations as in the optimal range, namely: 24°, 6-l. flow, 24 hr.; 24°, 15-l. flow, 24 hr.; 24°, 30-l. flow, 24 hr. Because the average percentage values for these three combinations are all nearly 100 (being 92, 95 and 95, respectively) it is possible to say that no other combination of influences could have given percentages significantly greater. Other equally effective combinations might of course be found, especially if the period of incubation were prolonged beyond 24 hr., but these three are surely as truly optimal as any combination that might be tested. Because all three of these optimal combinations are characterized by the temperature value of 24° we have said that the general temperature optimum for nearly complete germination is 24°. And because all three are characterized by the duration value of 24 hr. we may conclude that 24° and 24 hr. are to be taken together in defining a general environmental optimum, together with a rate of air flow of 6-l. per day or more, and the background conditions employed in this study.

(2) In the second place, from another point of view, we may regard as optimal the combination of experimental variables that gave the highest mean hourly rate of seedling production. On this basis, considering the duration factor now, we find that the optimal combination, within the whole range of this study, is 30°, 30-l. flow, 12 hr. This combination gave an average hourly rate of 6.2 seedlings per hour and no other combination tested approached that value more nearly than 5.0. It is apparent that no combination of these background conditions with a maintained temperature higher than 30° might have given a higher hourly rate, nor is there any suggestion that any incubation period shorter or longer than 12 hr. might have given higher rates, unless aeration were still more vigorous than is represented by an air flow of 30 l. per day. It is logically possible, however, that some rate of air flow more rapid than 30 l. per day might have given an average hourly rate of seedling production somewhat greater than 6.2. We may therefore conclude that the optimal complex for this lot of seed, within the parameter of this study, and on the basis of the time rate of seedling production, is either the one noted above or else some combination of 30° with a 12-hr. period and an air flow more rapid than 30 l. per day.

(3) From still another viewpoint, we may regard as optimal, in a special sense, the environmental complex corresponding to that particular portion

of the first 24 hours of incubation which gave the greatest 6-hr. increment in the numerical index of seedling production. On this basis we find an optimal set of conditions defined as follows: 30°, 30-l. flow, 2nd 6-hr. period. This complex gave an increment of 57 per cent. and no other combination tested approached that value at all closely. As by the second criterion (hourly rate), the optimal temperature is here shown as 30°, the optimal rate of air flow is to be considered as 30 l. per day, or else perhaps a still higher rate.

From these and similar considerations it is clear that the optimal temperature for seedling production by this lot of seed, under the background conditions of these studies, may be stated as about 24° or about 30°, according to the criteria used. Either statement may be regarded as correct. This illustrates the principle that no concept of optimal temperature, etc., can be generally clear unless the corresponding or concomitant non-temperature influences and the criteria for judgment are specifically stated.

Special experiments

The results of the routine experiments reported in the preceding section show many combinations of temperature, aeration treatment and length of incubation period that gave average germination percentages smaller than the largest value obtained, which was 95, for 24°, 24 hr. and an air flow of 15 l. or 30 l. per day. It is suggested at once that a large proportion of these low-efficiency combinations were deficient with respect to time only, that they might have given much larger germination percentages if the tests had been continued longer. Circumstances did not permit the longer-period tests that are thus suggested but two like experiments were carried out with an incubation period of 3 days, the seven maintained temperatures regularly employed, and an air flow of 15 l. per day, other details being according to the routine specifications. The average percentage values from these two experiments are given below, along with the corresponding 24-hr.-15-l. values and also the 24-hr.-30-l. values (from table I).

	12°	19°	24°	30°	35°	40°	45°
3-day values, air flow of 15-l. per day	95	95	95	87	28	13	14
1-day values, air flow of 15-l. per day	29	76	95	84	33	22	15
1-day values, air flow of 30-l. per day	29	82	95	93	45	21	15

It is remarkable that the incubation period of 3 days gave the same germination percentage for 12°, and 24° and that this value (95) is the same as the maximum percentage obtained with the combination of 24° and 15 l. or 30 l. and 1 day. It appears that this lot of seed was capable of showing a germination percentage of about 95 with temperatures from somewhat below 12° to about 24° (or perhaps even somewhat higher), with air flow of 15 l. or more, provided the incubation period were sufficiently prolonged. It is also indicated that the combination of 24° and air flow of 15 l. gave no more germination with a longer period than with a period of 1 day; with this combination it appears that all viable seeds had germinated by the end of the 1-day period. Just how long it took to attain the percentage value of 95 with combinations of 12° and 19° with air flow of 15 l. is of course not shown, but that value was surely attained within 3 days of the beginning of soaking. For 30° we may say that the 1-day period and the 3-day period gave about the same percentage value (87, 84) with air flow of 15 l. Whether the value for 1 day and a flow of 30 l. (93) is significantly higher than this may perhaps be questioned and it is at least possible that a period somewhat longer than 3 days might have brought the percentage value for 30° and a flow of 15 l. up to 95. There is obvious experimental discrepancy between the 1-day and the 3-day values for 35° and 40°, but it is at least indicated that all of the seeds that were able to germinate at either of these temperatures had burst their seedcoats by the end of the first day under the specified conditions; for this aeration treatment and either of these temperatures the percentage value is surely no greater for the 3-day period than for the 1-day period. The data for 1 day and 3 days with a temperature of 45° are in excellent agreement; as has been emphasized, all seeds capable of germination at this supra-optimal and surely injurious temperature had apparently burst their seedcoats within the first 6 hr. of incubation, the rest dying eventually.

Cultures held for 6 hr. at 45°, with an air flow of 15 l. per day, and then transferred to 24° with the same aeration for the following 18 hr. gave the same germination percentage as was shown for the first 6 hr. at the higher temperature; about 15 per cent. of the seeds germinated in the first 6-hr. period at 45° and no additional ones germinated in the succeeding 18 hr. at 24°. This supports the conclusion that the seeds that could not germinate in 6 hr. at 45° were probably all dead by the end of that short period.

From a special series of cultures of the sort used in the routine part of this study, with the same seven maintained temperatures and with an air flow of 3 l. per day, the seeds that had burst their seedcoats in the first 6 hr., in the second 6 hr., and in the second 12 hr. were segregated and planted in 21 pots of sand, which were kept in a greenhouse room for four weeks, with daily watering. Most of these lots of barely germinated seeds produced

nearly as many apparently healthy plants as there were seeds in the respective lots, indicating that the seeds had not been injured by the culture treatment employed for germination.

About half of the lot that had burst the seedcoats in 6 hr. at 45° failed to produce plants. This seems to indicate that about half of the germinated seeds from the 6-hr.-45° culture had been seriously injured in that short period, so that they either were dead at the end of the period or died without further development after they had been planted in the pots. The plants that were produced from this lot appeared to be somewhat retarded in their growth and may not have been quite as healthy as those from germination cultures at lower temperatures. Only a very few germinated seeds were available from the cultures at 45° for the second 6 hr. and for the second 12 hr. of incubation, and no plants were obtained from either of these lots. As far as this additional evidence goes, it supports the tentative conclusion reached from the results of the routine experiments, that a maintained temperature of 45°, with the non-temperature conditions of this study, acted injuriously on most of the seeds even in the first 6 hr.

Summary

In this paper are reported results of an experimental study of the germination performance of a lot of pure-line wheat seed (Nittany variety) under different sets of environmental conditions. The study was carried out at the Laboratory of Plant Physiology of the Johns Hopkins University, under guidance of Professor Burton E. Livingston. All apparently imperfect seeds and all seeds that floated on the nutrient solution used were discarded before each experiment was begun. In each germination test 100 seeds lay on the bottom of a germination flask, under 100 ml. of nutrient solution the surface of which was about 2 cm. above the seeds. Seven temperatures (12°, 19°, 24°, 30°, 35°, 40° and 45°) were employed by means of unlighted chambers, for incubation periods of 6 hr., 12 hr., 18 hr. and 24 hr. Six different aeration treatments were employed for each combination of temperature and length of incubation period. By one treatment the cultures were without air flow or agitation, the flask being open to the air of the chamber through an ordinary 2-hole rubber stopper. By the remaining treatments air from outside the temperature chamber was continually bubbled through the solution, renewing the supply of oxygen, removing volatile excretions and insuring the stirring of the solution about the seeds. Five rates of air flow were employed: 1 l., 3 l., 6 l., 15 l. and 30 l. per day. There were consequently 42 different environmental complexes, each being tested with four periods of incubation. The medium was a 3-salt solution containing per liter 0.0084 gram-mol. of each of the salts, KH_2PO_4, $MgSO_4$, $Ca(NO_3)_2$. It had a total osmotic value of about 1

atmosphere at 20°. The culture solution was not renewed throughout the incubation period. At the end of each test the number of seeds that had germinated was ascertained and recorded as a percentage of the total number in the flask. Germination was considered as having occurred in all instances where the seedcoat had been ruptured and the white coleoptile was visible through the opening. Each test was performed five times and the five percentages were combined as the average germination percentage for that test.

The results are presented in tabular form and in representative graphs of several sorts. Almost all (95, 92 per cent.) of the seeds of a sample germinated in 24 hr. with the best environmental complexes, and high percentages (86, 83 per cent.) were obtained in 18 hr. under the best conditions. About a fifth of them germinated in 6 hr. under the best conditions.

In general, higher temperatures gave higher germination percentages, up to a temperature optimum, beyond which still higher temperatures gave lower percentages. The optimal temperature was lower as the incubation period was longer, being about 30° or 35° for the shortest period and about 24° for the longest. Aeration treatment exerted little if any influence on the optimal temperature for any period.

For 12°, 19°, 24° and 30° the germination percentages secured with any aeration treatment were progressively higher with longer incubation periods and the highest values were obtained with these temperatures and 24 hr. of incubation. On the other hand, this relation was not so clear for still higher temperatures and did not appear at all for 45°. The seeds that germinated at this highest temperature burst their seedcoats within 6 hr. and longer periods gave no higher values. Aeration treatment was without marked influence on this relation.

In general, progressively more vigorous aeration treatments gave progressively higher germination percentages, up to a rate of air flow of 3 l. or 6 l. per day, but no farther, and this relation was increasingly evident for progressively longer periods. For the longest period (24 hr.) temperatures of 19°, 30° and 35° gave progressively higher values up to the most rapid rate of air flow (30 l. per day) and it appears that still more vigorous aeration might have given higher values than were given by the 30-l. flow. With a temperature of 24° a double aeration optimum is clearly shown for the 18-hr. and 24-hr. periods and such a double optimum is suggested for 19° and 24 hr. and for 19° and 18 hr. The bimodal aeration graph representing this double optimum has a marked secondary minimum corresponding to an air flow of 3 l. per day. That is, for the instances just mentioned rates of flow of 1 l. and of 6 l. per day gave notably higher percentages than were given by the intermediate rate of 3 l. per day.

Some representative 3-dimension graphs are shown, with contours or isopleths passing through points that represent combinations of conditions giving like percentage values.

Average hourly rates of seedling production are presented and discussed, also the 6-hr. increments of average germination percentage.

As to optimal conditional-durational complexes, three complexes gave percentage values above 90, all for 24° and 24 hr., with air flow of 6 l., 15 l. and 30 l., respectively. These combinations of the experimental variables might be taken to represent general optimal conditions. If, however, we consider as optimal the combinations that gave highest average hourly rates of seedling production we must regard as optimal the combination of 30°, 12 hr. and an air flow of 30 l. per day. This gave an average hourly rate of 6.2 seedlings per day and no other combination tested gave a corresponding hourly value approaching this more nearly than 5.0. Furthermore, if we regard as optimal the complex corresponding to that particular 6-hr. interval (of the first 24 hr. of incubation) which gave the greatest 6-hr. increment in the numerical index of seedling production, we should select the second 6-hr. period with 30° and an air flow of 30 l. per day. This complex gave an hourly increment of 57 per cent. which is not at all closely approached by any other corresponding value.

Some special experiments are briefly reported. From an experiment series with an incubation period of 3 days it appears that this lot of wheat seed was capable of showing a germination percentage of about 95 with temperatures from somewhat below 12° to about 24° (or perhaps higher), with daily air flow of 15 l. or more, provided the incubation period were sufficiently prolonged. This may be true for 30° also. For 35° and for 40°, with air flow of 15 l. the 3-day cultures failed to give greater percentage values than were given by single-day cultures. For 45° no more seeds germinated in 3 days than in 6 hr.

Cultures held for 6 hr. at 45° with air flow of 15 l. per day and then subjected to 24° with the same aeration for the succeeding 18 hr., gave a germination percentage of about 15, the same as was given by the first 6 hr. of this test. This supports the conclusion that the seeds that could not germinate in 6 hr. at 45° were probably all dead by the end of that short period.

Seeds that had germinated in the first 6 hr., in the second 6 hr. and in the second 12 hr. of incubation, with air flow of 3 l. per day, and had then been planted in pots of sand and treated for four weeks like ordinary potted plants in the greenhouse, generally showed healthy and usual growth, which indicates that the germination treatment had not been injurious. But about half of the seeds that had germinated in the first 6 hr. at 45° (with 3-l. air flow) failed to continue their development in the pot cultures;

these had been killed in the 6 hr. germination period or else they had been so injured that they died shortly after being planted in the pots.

The results of this study clearly show how the germination performance of this lot of wheat seed was influenced by the experimental variables (maintained temperature, aeration treatment and duration of incubation) and how the influence exerted by any one of these variables was itself influenced by the others. They emphasize again the general principle that environmental influence on organisms needs to be studied and described in terms of environmental complexes rather than in terms of only one or a few of the influential conditions. The influence of any kind of environmental condition or "factor" can be usefully described only with adequate reference to all the other kinds of influential conditions acting at the same time.

It is pointed out that germination with a series of different but simultaneous environmental complexes and different incubation periods might be employed to secure several batches of barely germinated seeds from the same seed stock, the individuals of each batch being rather nearly alike physiologically while the several batches might be nearly alike or notably different, as might be desired. When like batches of seedlings from a given seed stock are needed for experimentation on environmental influences as they affect subsequent devlopmental phases, such physiological selection as is here suggested may be useful, in addition to the commonly employed selections based on general appearance, size, color, weight, specific gravity, and other morphological characteristics of resting seeds.

The relations here brought out between germination percentage, on the one hand, and the prevailing environmental complexes of the germinators and the duration of incubation, on the other hand, may be useful also in the further working out of standard procedures for ordinary seed testing.

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DISCHARGE OF SACCHARASE FROM MYCELIUM OF *PENICILLIUM GLAUCUM*

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(WITH TWO FIGURES AND ONE PLATE)

Introduction

The relation between the composition of the media of fungus cultures and the enzymes produced has been the subject of many investigations. There is very little known about the discharge of the enzymes secreted by the mold into the culture medium. This phenomenon bears the most intimate relationship to the rôle played by the enzymes and to the life-cycle of the molds and it therefore merits more attention.

The first recorded data in the literature that deal with the diffusion of mold enzymes into the medium are found in the study of FERNBACH (**7**) on the saccharase of *Aspergillus niger*. His method can not be regarded as free from objections, (**5**) but he established the first enzyme unit and he was the first to point out the great importance of the degree of acidity of the reaction mixtures. He measured the distribution of the saccharase between the mold and medium. The main result of his investigations was the discovery of the fact that the total amount of saccharase (activity) expressed in his enzyme unit was fairly constant, decreasing only about 20 per cent. from the second to the fifth day. The distribution ratio of the enzyme between the mold and medium changed during growth. On the second day 3 per cent., and on the fifth day 37 per cent. of the total enzyme content was found in the medium.

The conclusion to be derived from FERNBACH's experiment is that the maximum amount of enzyme is formed during the first two days, and later this enzyme simply passes slowly from the mold to the medium. He obtained practically the same results with a yeast,—*Saccharomyces pastorianus*. Since FERNBACH's experiments no work has been done on the distribution of the enzyme between microorganism and medium.

In the experiments of DOBY and KERTESZ (**2**) the saccharase content of *Penicillium glaucum* cultures was determined and the enzyme content of the medium was found to be very low. These authors were studying the changes in the saccharase content of the mold when grown with and without potassium, and therefore did not pay further attention to the enzyme content of the medium.

BRIDEL and AAGAARD (**1**) have shown that when the mold is kept on distilled water, the enzyme diffuses out of the mycelium into the water.

IWANOFF and KUDRJAWZEWA (**8**) published a paper in 1929 entitled,

"Ausscheidung der Saccharase aus der Zellen." The main conclusions drawn by these authors were that the discharge of the saccharase from the mycelium depends on the pH of the medium; very little saccharase was discharged if the medium was acid but as it became more alkaline more and more enzyme was discharged into the medium. In this connection it should be pointed out that one essential weakness of the method used by the Russian authors is that the saccharase content of the medium only has been studied and never that of the mold. For this reason the data that these workers presented can not be considered sufficient to show completely the extent of the discharge of the saccharase. Although the enzyme content (capacity) of the medium of two different cultures may be found to be the same, this part of the enzyme content may be only 10 per cent. in one case but 90 per cent. of the total enzyme content produced by the mold in the other case.

It was for the purpose of securing more information concerning the factors influencing the discharge (diffusion) of saccharase from the cells of *Penicillium glaucum* that the studies reported in this paper were undertaken.

Experimental

Method

The strain of *Penicillium glaucum* Link used in these experiments was the same as used in earlier experiments, the culture originating from the Royal Hungarian Institution for Industrial Fermentations in Budapest, Hungary.

The salt supplement of the cultures was always the same as given in earlier papers (**10, 11, 2**) and contained sufficient amounts of P, K, Mg, Ca, Na, Cl, SO_4, N, (NO_3 and H_4 N) and traces of Fe^{+++} and Zn. After inoculation the mold has been grown in 100-cc. Erlenmeyer flasks containing 50 cc. of medium and at a temperature of 24.0° C.

The procedure used in the enzyme determination was as follows: The mold was taken off of the medium and washed with a quantity of distilled water such that the volume of the medium was restored to 50 cc. as at the beginning of the growth. The dry matter was determined on a small sample of mycelium, drying to constant weight at 95° C. The rest of the mold was ground in a porcelain mortar and suspended in water. A sample of this suspension was taken for the determination of the dry matter content of the suspension and the remainder was used for the determination of the enzyme activity.

The medium was filtered before being used for the determination of the enzyme activity. The reaction mixtures contained in all cases 5 per cent. of sucrose. The amount of sucrose contained in the part of the medium

used has always been determined and correction for this has been made in the calculations. The pH of the reaction mixture was always 4.5 which has been shown to be the optimum for this enzyme (**2**). The pH was determined by the use of indicators. Phosphate buffer was used throughout and 3 per cent. of toluol was always added. The reaction temperature was 38.0° C. in all experiments.

At the beginning of the experiments and at the indicated intervals, samples were taken from the reaction mixtures, clarified by neutral lead acetate and sodium carbonate, centrifuged, and in the clear solution the reducing sugars were determined. In one series the optical rotation was determined in a 200 mm. tube. For the determination of the reducing sugars Bertrand's method was used and for the calculation reference was made to the author's recalculated tables (**12**). The values reported in the tables have been corrected for the blank found at the beginning of the experiment.

The monomolecular reaction constants were calculated by the use of the familiar formulae:

$$k = \frac{1}{t \times 0.4343} \cdot \log \frac{A}{A - X} \tag{1}$$

and

$$k = \frac{1}{t \times 0.4343} \cdot \log \frac{\alpha_0 - \alpha_\infty}{\alpha - \alpha_\infty} \tag{2}$$

in which "t" is reaction time (min.), A is the amount of substrate (sucrose) present at the beginning of the reaction, X is the amount of substrate changed in time "t," α_0 is rotation at the beginning, α is rotation after the time "t." The value of α_∞ is calculated from the formula

$$\alpha_\infty = \alpha_0 \, (0.417 - 0.05\, t). \tag{3}$$

The If is calculated from v. EULER's formula (**6**)

$$If = \frac{k \times \text{gm. sucrose in the reaction mixture}}{\text{gm. of dry matter of the enzyme preparation in the reaction mixture}} \tag{4}$$

In the case of the enzyme determinations in the medium "cc. of medium used in the reaction mixture" has been substituted for "gm. dry matter."

The enzyme content of the whole culture of the mold and medium respectively has been expressed in the formula proposed by the author (**13**):

$$\text{Total enzyme content of the mold} = E_1 = (If_1) \times (\text{dry matter yield in gm.}) \tag{5}$$

$$\text{Total enzyme content of the medium} = E_2 = (If_2) \times (\text{total volume of the medium in cc.}) \tag{6}$$

Before presenting the data obtained, it is necessary to explain why the above method of determination has been used in preference to the method described by IWANOFF and KUDRJAWZEWA. As mentioned before, the Russian authors determined the enzyme content in the medium only. Further, the method used for their determinations was not free from objections. Their method as described in their paper was as follows (*loc.*

cit., p. 243): "Von 50 ccm. Kulturflüssigkeit wurden 10 ccm. Filtrat entnommen und 10 ccm. Wasser und 1 gm. Saccharose hinzugefügt; das Medium wurde auf pH = 5.2, t = 52° C. gebracht; nach Verlauf einer bestimmten Zeit, 1 bis 3 Stunden, wurde die Menge des reduzierenden Zuckers nach Bertrand bestimmt. Die Saccharasekraft wurde in Milligrammen reduzierenden Zuckers ausgedrückt, welcher sich aus 1 gm. Saccharose gebildet hat. Diese einfache Methode wurde bei allen weiter beschriebenen angewandt."

It is to be seen that no determinations of the reducing power were made at the beginning of the reactions. The media used by IWANOFF and KUDRJAWZEWA contained sugars in most cases and perhaps other substances which have a reducing power; therefore their single determination of the reducing power could scarcely be expected to define the rate of reaction. If the mold was grown, for instance, on sucrose solution the original reducing power of the medium was changed considerably by the inversion of the sucrose. These two opposing reactions (1) the inversion of the original sucrose, and (2) the disappearance of the invert sugar from the solution, make the results obtained by the Russian authors so uncertain, that from their work no more than qualitative conclusions can be drawn.

RESULTS

On the basis of the experiments of IWANOFF and KUDRJAWZEWA attention was first paid to the influence of the reaction of the medium on the discharge of the saccharase to the medium.

In these experiments the *Penicillium glaucum* was grown on 50 cc. of medium containing 5 per cent. sucrose and inorganic salts. By the use of N/10 NaOH or N/10 H_2SO_4 the reaction of the medium was brought to the desired pH. A few drops of solutions of suitable indicators were added and the pH corrected daily throughout the experiment by the addition of base from a sterile burette. Of course, during one day the production of acid by the living mold caused a certain shifting toward the acid reaction, therefore the pH values given are approximately 0.5 pH higher than the lowest value actually reached during growth. The cultures were harvested on the fourth day after inoculation. In this single case the determination of the saccharase effect in the mold was done by the polarimetric method.

The results obtained show that contrary to the conclusions stated by IWANOFF and KUDRJAWZEWA, saccharase has been found in the media of cultures grown in acid reaction. On the whole the total enzyme content ($E_1 + E_2$) decreased with increasing pH. The pH of the first culture was 3.0 at the beginning, and it was necessary during growth to correct it, because it had decreased to 2.7 – 2.8. From this experiment it is to be seen that the saccharase of *Penicillium glaucum* passes into the medium in quite acid reaction.

TABLE I

SACCHARASE CONTENT OF THE MYCELIUM AND MEDIUM OF *Penicillium glaucum* CULTURES, GROWN AT DIFFERENT pH 'S ON 5 PER CENT. SUCROSE FOR 4 DAYS

pH OF THE MEDIA	DRY MATTER YIELD	SACCHARASE IN THE MOLD				SACCHARASE IN THE MEDIUM		$(E_1 + E_2) \times 10^3$	TOTAL SACCHARASE IN THE MEDIUM
		DRY MATTER IN THE DETERMINATION	AVERAGE $k \times 10^4$	$If \times 10^3$	$E_1 \times 10^3$	AVERAGE $k \times 10^4$	$E_2 \times 10^3$		
	gm.	*gm.*							*per cent.*
3.0	0.115	0.1319	10.61	24.13	3.74	0.37	0.26	4.00	6.5
4.1	0.111	0.0968	9.19	27.57	3.06	0.59	0.41	3.47	11.8
5.1	0.067	0.0549	4.03	22.02	1.48	0.51	0.36	1.84	19.6
6.3	0.043	0.0383	6.79	53.20	2.28	1.46	1.02	3.30	30.9
7.0	0.026	0.0214	2.11	29.57	0.77	0.45	0.31	1.08	28.7
7.9	0.028	0.0236	2.83	35.94	1.01	0.23	0.16	1.17	13.7
8.3	0.025	Not determined				0.40	0.24	(0.24)	
9.8	Fragments of mycelium					0.26	0.18	(0.18)	

TABLE II

SACCHARASE CONTENT OF THE MYCELIUM AND MEDIUM OF *Penicillium glaucum* GROWN ON 50 CC. OF 5 PER CENT. SUCROSE SOLUTION AND INORGANIC SALTS

AGE	ACIDITY OF THE MEDIUM	SACCHARASE IN THE MOLD						SACCHARASE IN THE MEDIUM					$(E_1 + E_2) \times 10^3$	TOTAL SACCHARASE IN THE MEDIUM
		DRY MATTER IN EXPERIMENT	INCREASE IN INVERT SUGAR IN 6 HOURS	$k \times 10^4$	$If \times 10^3$	DRY MATTER YIELD	$E_1 \times 10^3$	USED FOR THE DETERMINATION	INCREASE IN INVERT SUGAR IN 6 HOURS	$k \times 10^4$	SUCROSE IN THE MEDIUM	$E_2 \times 10^3$		
days	*pH*	*gm.*	*mg.*			*gm.*		*cc.*	*mg.*		*per cent.*			*per cent.*
2	2.9	0.0406	7.5	2.06	15.22	0.057	0.87	30	0.0	.....	3.80	0.0	0.87	0.0
4	2.8	0.1746	58.1	22.40	38.50	0.396	15.25	30	22.4	5.69	1.59	3.29	18.54	17.7
7	2.8	0.4340	20.1	5.91	4.09	0.662	2.71	30	4.2	0.10	1.56	0.06	2.77	2.1
11	2.6	0.3187	2.3	2.58	2.43	0.857	2.13	35	0.2	0.05	1.34	0.02	2.15	1.0
16	2.6	0.3000	8.0	2.20	2.20	1.014	2.23	30	0.7	0.17	0.70	0.09	2.32	3.8

The rate of growth, as is to be seen from the dry matter yield, varied materially in the cultures grown at different pH's. All the cultures were therefore in different stages of development, and presumably some of them near to and some far from having their highest saccharase content. DOBY and KERTESZ found that the mycelium of *Penicillium glaucum* grown on 5 per cent. sucrose had the highest saccharase content on the fifth day of growth. This work showed the effect of pH on the growth of the mold, but no attempt was made to arrive at conclusions in regard to the distribution of the enzyme between mycelium and medium. For this reason the changes of the saccharase content of growing cultures were studied as will be shown later in this paper.

In the following three experimental series given in tables II, III and IV the mold was grown on 50 cc. of a medium which contained the inorganic salt supplement and 5 per cent. of sucrose (II), the salt supplement and 5 per cent. of sucrose and 0.25 per cent. of asparagin (III), and the salt supplement and 1.5 per cent. solution of Witte peptone (IV).

As can be seen from table II, the dry matter yield increased even after the eleventh day, showing that the supply of nutrients had not been used up during that time. The enzyme content of both mycelium and medium was the highest on the fourth day. The highest enzyme content of the medium was observed on the fourth day and amounted to 17.7 per cent. of the total enzyme content.

As was seen from the pH values of the growing cultures, the reaction of the medium was shifted toward the acid side very quickly, *i.e.*, the original reaction of pH 5.4 was changed to 2.9.

The results presented in table III are similar to those presented in table II. The growth is more rapid, and because of the larger amount of nitrogenous and carbohydrate nutrients the dry matter yield is higher also. The total enzyme (or enzyme activity) produced by the culture at the fourth day is double that of the culture having no asparagin. The rate of increase of the actual acidity in the medium is a little slower in the presence of asparagin, possibly because of the buffer action of this compound.

The maximum enzyme activity per unit of dry matter (If_1) is only 10 per cent. higher than in the culture without asparagin, but the enzyme content of the medium is much higher. But even in this case only on the fourth day was 25 per cent. of the total enzyme content found in the medium; later this value decreased to 5 per cent., only one twentieth of the total enzyme contained in the whole culture.

The results obtained with cultures grown on Witte peptone are presented in table IV. In no case has a definite saccharase activity been found.

TABLE III

SACCHARASE CONTENT OF THE MYCELIUM AND MEDIUM OF *Penicillium glaucum* GROWN ON 50 CC. OF 5 PER CENT. SUCROSE, 0.25 PER CENT. ASPARAGIN, AND INORGANIC SALTS

AGE	ACIDITY OF THE MEDIUM	SACCHARASE IN THE MOLD						SACCHARASE IN THE MEDIUM						
		DRY MATTER IN THE DETERMINATION	INCREASE IN INVERT SUGAR IN 6 HOURS	$k \times 10^4$	$If \times 10^3$	DRY MATTER YIELD	$E_1 \times 10^3$	USED FOR THE DETERMINATION	INCREASE IN INVERT SUGAR IN 6 HOURS	$k \times 10^4$	SUCROSE IN THE MEDIUM	$E_2 \times 10^3$	$(E_1 + E_2) \times 10^3$	TOTAL SACCHARASE IN THE MEDIUM
days	*pH*	*gm.*	*mg.*			*gm.*		*cc.*	*mg.*		*per cent.*			*per cent.*
2	4.4	0.1010	15.0	4.29	12.74	0.141	1.33	30	0.3	0.06	3.41	0.04	1.37	3.3
4	2.9	0.2950	75.4	41.62	42.40	0.703	29.3	30	51.3	18.64	0.44	9.73	39.03	24.7
7	2.7	0.9048	36.9	11.71	38.91	1.279	4.97	30	17.4	5.04	0.29	0.26	5.23	5.0
11	2.7	0.3958	21.4	6.34	4.80	1.268	6.08	35	24.0	7.22	0.44	0.32	6.40	5.0
16	2.7	0.3010	15.6	4.47	4.46	1.498	6.68	30	26.5	8.08	0.32	0.42	7.10	5.9

TABLE IV

SACCHARASE CONTENT OF THE MYCELIUM AND MEDIUM OF *Penicillium glaucum* GROWN ON WHITE PEPTONE AND INORGANIC SALTS

AGE	ACIDITY OF MEDIUM	SACCHARASE IN THE MOLD							SACCHARASE IN THE MEDIUM							PRESENCE OF ENZYME DETERMINED BY THE			
		DETERMINATION AFTER HOURS							DETERMINATION AFTER HOURS							ROTATION METHOD		REDUCING METHOD	
		DRY MATTER IN EXPERIMENT	0		6		24		USED FOR THE DETERMINATION	0		6		24		MOLD	MEDIUM	MOLD	MEDIUM
			ROTATION	INVERT SUGAR	ROTATION	INVERT SUGAR	ROTATION	INVERT SUGAR		ROTATION	INVERT SUGAR	ROTATION	INVERT SUGAR	ROTATION	INVERT SUGAR				
days	*pH*	*gm.*	*deg.*	*mg.*	*deg.*	*mg.*	*deg.*	*mg.*	*cc.*	*deg.*	*mg.*	*deg.*	*mg.*	*deg.*	*mg.*				
2	6.9	.....	.....	.....	.....	.....	.....	.....	30	0.398	3.3	0.404	.....	0.422	2.0	(+)	–	–	–
4	7.4	0.037	0.552	2.6	0.540	2.6	0.514	1.1	30	0.339	3.3	0.474	.....	0.458	1.7	(+ ?)	–	(+)	–
7	7.6	0.084	0.474	2.9	0.510	3.1	0.425	3.4	30	0.452	2.6	0.434	2.6	0.458	1.8	–	–	+	–
11	7.4	0.062	0.524	1.9	.....	.....	0.550	3.0	30	0.418	2.0	0.396	.....	0.402	4.0	–	–	+	+

It is to be seen from the data presented that the question of the diffusion of saccharase to the medium in mold cultures is much more complicated than it has been supposed by earlier authors. With changes in the medium, changes occur in the whole life-cycle of the mold. On account of the different substrates, the molds produce different amounts (activity) of enzyme. Furthermore the enzyme content changes with the age of the culture.

In the following experiment mold grown on 5 per cent. sucrose for five days was kept immersed in buffer solutions of various pH in the presence and also in the absence of toluol and the enzyme diffused to the buffer solutions after two days was determined. The mold had been grown in five 100-cc. Erlenmeyer flasks each containing 50 cc. of medium. The weight of mycelium harvested on the fifth day was 13.75 gm. The dry matter content was determined to be 19.15 per cent. The total dry matter yield of the five cultures was therefore 2.63 gm. After washing and drying with filter paper it was divided into 1-gm. portions each representing 0.19 gm. dry matter. Ten of these portions were put in small bottles having glass stoppers and containing 40 cc. of a mixture of 4 cc. N/10 phosphate buffer and 36 cc. of sterilized distilled water making a final buffer solution N/100 with respect to phosphate. The mold was immersed in the solutions. At the beginning of the experiment the enzyme content (activity) of the mold was determined to be $If = 31.6 \times 10^{-3}$. For the determination of the enzyme diffused to the solutions, after filtration 25 cc. was mixed with 20 cc. of a 12.5 per cent. sucrose solution and 5 cc. of phosphate buffer, pH = 4.5. In several cases the solutions were too far removed from pH 4.5 to use directly and in these the pH was corrected by the addition of N/20 NaOH or N/20 H_2SO_4. From these reaction mixtures 10 cc. was taken out at the intervals indicated, clarified by neutral lead acetate and sodium carbonate, and made up to 100 cc. This solution was used for the determination of the reducing sugars produced by the action of the saccharase. In figure 1 are shown the results of these experiments.

In all these last experiments containing buffer solutions the enzyme activity was higher than in the blank in which the mold had been kept on sterile distilled water. The shape of the curves representing the relations between pH of the solutions in which the mold was kept and the enzyme found in it after two days are very remarkable. In the presence of toluol the highest enzyme content was found in the neutral solution, while in the absence of toluol the lowest enzyme content was found at the same reaction. The highest enzyme content recorded was found in the solution without toluol at pH 8.9. In this case the solution contained 2.8 times as much enzyme as the blank on distilled water.

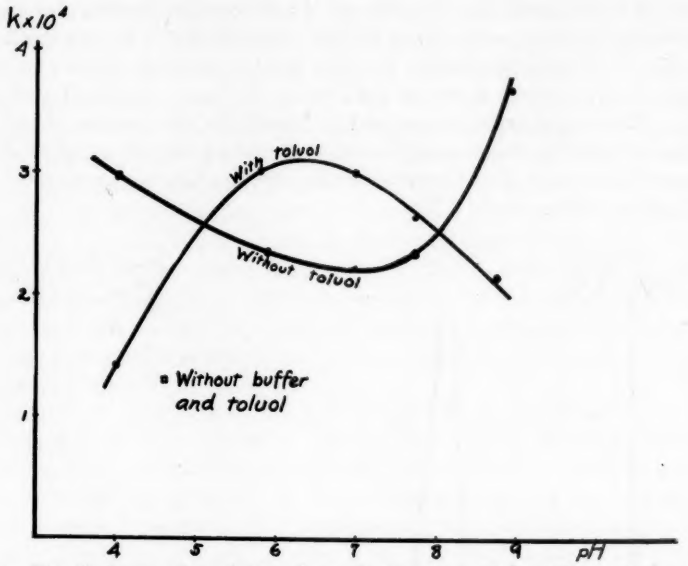

FIG. 1. The discharge of saccharase from the mycelium of *Penicillium glaucum* cultures. (The k's are averages of the values obtained in 3, 6 and 24 hours.)

Discussion

A great deal of work has been done on the permeability of the plant cells, and on the factors influencing the diffusion of different plant materials, but among the many theories presented no one theory can be found to explain this phenomenon satisfactorily. It has been supposed that in the diffusion of different compounds through the cell wall, the VAN'T HOFF rule is not applicable. It was supposed furthermore, that besides the osmotic permeability there exists "selective permeability" (**15**), which would permit the diffusion of compounds like sugars and some amino-acids. It has been observed that toxic materials of presumably high molecular weight are put out from cells which contain concentrated solutions of compounds of low molecular weight, but which do not pass out of the cell at all. The passage of enzymes through cell walls is no doubt a complicated matter. A few papers dealing with the diffusion of enzymes through collodion and similar membranes have been published but very little has been done on the diffusion through cell walls.

In an earlier paper the author pointed out that the formation of enzymes bore a most intimate relationship with the necessity of the organism for them (**10**). In the case of a sucrose medium the saccharase was presumably

produced by the mold for the purpose of splitting the sucrose into simpler compounds which possibly could be more easily utilized by the mold than sucrose. It is entirely possible that the mold is not able to use sucrose at all, since in the case of a sucrose medium saccharase is produced quite generally. If the saccharase is produced by the cell for the purpose of splitting sucrose it is easy to understand that the amount of enzyme required should decrease after most of the sucrose of the medium has been converted into invert sugar (**11**).

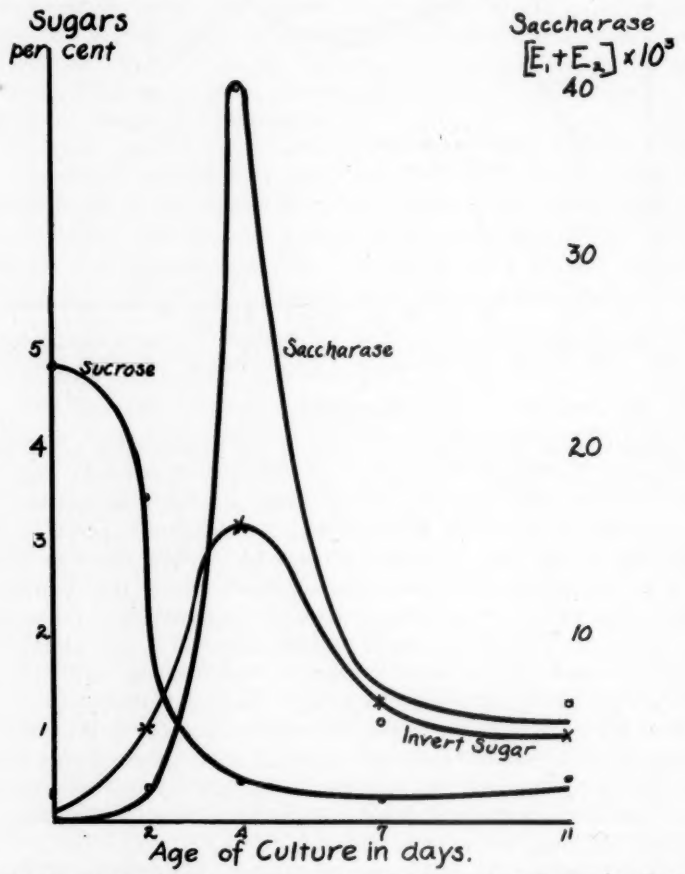

FIG. 2. The relation between sucrose and saccharase content of growing *Penicillium glaucum* cultures. (Grown on 50 cc. of 5 per cent. sucrose solution, containing 0.25 per cent. asparagin and inorganic salts.)

It is to be seen from fig. 2 that the greater part of the sucrose of the medium was inverted in the interval between the second and fourth day. In fact 63 per cent. of the total sucrose content of the medium had disappeared during this period. It is also to be seen that the saccharase content of the cultures was the highest at the fourth day of growth. After the greater part of the sucrose was converted into invert sugar the saccharase content decreased abruptly. It is interesting to note that a small amount of sucrose (or another polysaccharide?) is present even after sixteen days. A very low saccharase activity is to be found at this same time.

The results of the present experiments are not in harmony with those of the earlier investigators. Of course, the subject of their experiments has not been *Penicillium* but *Aspergillus niger,* but the author is convinced that the difference is caused more by the method of investigation than by the organism used.

The pH of the media in the experimental series in table II has been determined to be 2.9 on the second day. The discharge of the enzyme however does not start until later, since on the second day no enzyme was found in the medium. This fact is in very good harmony with the results presented in table I, where with the cultures grown on acid media, saccharase has been found in the nutrient solution. IWANOFF and KUDRJAWZEWA (p. 246) state that if cultures have been grown on a medium containing sucrose for four days, and to the medium oxalic acid is then added, no saccharase effect could be found after two or eight days.

The writer, in an attempt to show this directly, tried to grow cultures (*Penicillium* and *Aspergillus niger*) in the presence of oxalic acid in the amounts used by the Russian authors but no growth could be obtained. The reaction of these solutions has been determined to be around pH 1.25–1.70. Whatever saccharase is discharged to such a solution would lose its activity entirely in a very short time, because this high acid reaction has been shown to be very toxic to saccharase (**4**). NELSON and PALMER (**14**) observed that yeast saccharase is affected by a reaction of pH 4.6. Furthermore, oxalic acid itself is known to be one of the most toxic of organic acids for molds (**9**). With these observations of others in mind, it is obviously impossible to say from the experiments of IWANOFF and KUDRJAWZEWA that the acidity alone of the medium used has prevented the saccharase from passing out of the mycelium into the medium. Even if any enzyme should pass out, it would shortly become inactivated due to the high acidity, aggravated in this case by the toxic oxalate ion.

In the experiments with growing cultures presented in this paper the reaction of the medium always turned acid with the exception of the experiments with the peptone medium, where no definite saccharase effect could be obtained. It is to be seen from the first series, that the medium

of cultures grown at a pH of 6.3, 7.0 and 7.9 contained as much as 30 per cent. of the total saccharase content of the whole culture. The question whether this saccharase is discharged by the normal diffusion of uninjured cells or whether it is coming from the cells killed by the alkaline medium can not be decided from these experiments.

To be able to make a study of the discharge at different pH reactions, without being disturbed by the different rates of growth of the mold, an experimental series has been carried out, the results of which are presented graphically in fig. 1. The results obtained are no doubt due to the operation of a great many factors. The different parts of the curves should be explained in quite different ways, since the influence of the acid and alkaline medium is not identical.

From the first experimental series as well as from these later experiments it can be seen that the mold without toluol discharges a great deal of its enzyme content to an alkaline medium. This observation is in harmony with IWANOFF and KUDRJAWZEWA'S results, who found that *Aspergillus niger* always put out more enzyme on an alkaline than on a neutral medium. On the acid side of this curve enzyme activity could be observed also. This is no doubt the result of a certain growth continued by the mold on the buffer solution. At the neutral reaction, a lower enzyme activity has been found. This reaction is certainly not favorable for the growth of the mold neither does it cause an increase of the enzyme by discharge nor by the plasmolysis of the cells.

Quite different are the results of the experiments in the presence of toluol. If the mycelium of the mold is placed into buffer solutions on which there is some toluol, the toluol is in much more intimate contact with the mold than with a suspension of the mycelium in the autolytic experiments of DOBY and KERTESZ. VON EULER and B. VON EULER-AF UGGLAS (**3**) established the fact, that the action of different protoplasmic poisons is quite different on living and non-living cells. They found that toluol killed the saccharase of *Monilia,* if living cells were exposed to it, but had only a very little effect, if dead (dried) cells were treated. The experiments here described show further that toluol is more toxic to the saccharase at either acid or alkaline reaction than at neutral reaction.

To make a further study of the effect of different treatments on the cells of *Penicillium,* microscopic sections have been prepared.[1] Photographs (x 80) of some of these are presented on the accompanying plate VIII. No. I is taken from a section prepared from the mold which had stood on an acid buffer solution for two days without toluol. It can be seen that there is a secondary growth quite distinct from the mycelium which had been

[1] The writer's sincere thanks are due to Dr. MABEL NEBEL and to Dr. BERNARD R. NEBEL for preparing these sections and photomicrographs.

formed on the original medium. There is also a rich spore formation to be seen. No. II is prepared from the mold from the alkaline solution without toluol. The mycelium and the layer of spores are wrinkled and the hyphae are partly empty as a result of the effect of the alkalinity. In this sample the greatest discharge of the saccharase had been found. Plate VIII no. III is taken from the section prepared from the mold from acid solution with toluol. The structure in general is very badly affected by the toluol. No secondary growth can be seen and no plasma can be observed in the sporangia. The mold in alkaline solution and in the presence of toluol is even more drastically affected (no. IV).

No doubt the present state of our knowledge is not sufficient to draw general conclusions in regard to the discharge or diffusion of enzymes. This can be done only after collecting more data and making further studies of this interesting as well as important phenomenon.

Summary

1. The discharge of saccharase in growing cultures of *Penicillium glaucum* has been studied. The highest enzyme content of the mycelium and medium has been found to occur on the fourth day of growth. Never more than one third of the total enzyme content of the culture has been found in the medium.

2. The saccharase can pass into the culture medium even at acid reaction, but the rate of discharge is lower in an acid than in an alkaline medium.

3. A marked decrease of the enzyme content of both mold and medium has been observed after most of the sucrose present in the medium was inverted.

4. When the mycelium of five-day-old mold containing saccharase ($If = 31.6 \times 10^{-3}$) had been kept on a series of buffer solutions of pH's 4.0–8.7, saccharase was detected in solution after two days in all cases.

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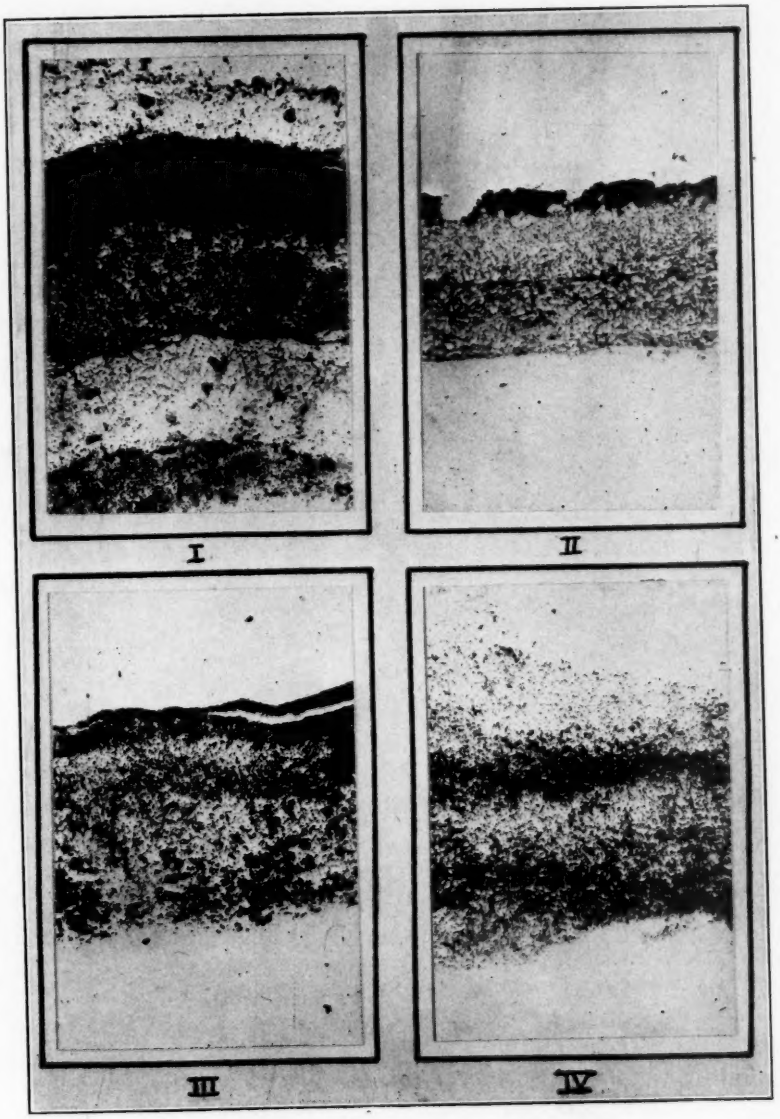
EXPLANATION OF PLATE VIII

No. I. Section from five days old *Penicillium glaucum*, which stood in acid buffer solution (pH = 4.1) for two days.

No. II. Section from five days old *Penicillium glaucum* which stood in alkaline buffer solution (pH = 8.9) for two days.

No. III. Section from five days old *Penicillium glaucum* which stood in acid buffer solution (pH = 4.0) in the presence of toluol for two days.

No. IV. Section from five days old *Penicillium glaucum* which stood in alkaline buffer solution (pH = 8.7) in the presence of toluol for two days.



KERTESZ—DISCHARGE OF SACCHARASE

LIGHT AND THE CAROTINOID CONTENT OF CERTAIN FRUITS AND VEGETABLES

LAURA LEE W. SMITH AND ORA SMITH*

Introduction

While studying the relationship of vitamin A and carotinoid content of fruits ripened under controlled conditions, it became evident that a consideration of this problem would not be complete without a study of the light factors involved. The object of this investigation was to find out, if possible, the effect of the exclusion of light from the developing fruits of various fruits and vegetables on their carotinoid content. That portion of the study of the relation of vitamin A to the carotinoid content will be presented elsewhere in a separate paper.

A search of the literature failed to disclose any work done on the effects of complete exclusion of light from the fruits of plants on their carotinoid content.

OVERHOLSER (**12**), interested primarily in anthocyanin formation, excluded light from the fruits of apples, pears, peaches, apricots and nectarines by means of black sacks and found that, in most varieties, no red color developed. The fruits were bagged, however, after chlorophyll had developed in them.

Material and methods

The following kinds and varieties of fruits were selected for their differences in flesh and skin color and in pigment content: Elberta and Mayflower peaches, Humboldt and Stanwick nectarines, Royal apricots, Clark's Albino, Ruby Gold, Globe, California Earliana and Gigante Ingregnoli varieties of tomato.

PREPARATION OF TREE FRUIT SAMPLES

The Elberta is a deep yellow-fleshed peach; the Mayflower an early white-fleshed peach; the Humboldt a yellow-fleshed nectarine; the Stanwick a white-fleshed nectarine and the Royal a deep yellow-orange-fleshed apricot. These fruits were selected from the station orchards at University Farm, Davis, California.

On March 31, 1928, the flowers and fruit were bagged with manila bags lined with either a heavy sized black paper (skytogen) or a double thickness of high grade thin black paper. The apricots and Elberta peaches

* These investigations were conducted at the University of California while the senior author was a member of the Household Science Department and the junior author connected with the Division of Truck Crops.

had set at the time of bagging, the former being about one centimeter in diameter and the latter about 4 millimeters in diameter; the petals of the nectarines and white peaches were beginning to fall. The bags were tied on over clusters of fruit and blossoms and not removed until harvested. The loss from drop was quite heavy with the Elberta peach and the Stanwick nectarine, and it was necessary to bag fruit again during the early part of May. Bagged fruits and a similar quantity of unbagged fruits from the same location on the tree were harvested at the same time.

The fruits were peeled, seeded, cut into small pieces and placed in small air-tight non-actinic glass bottles with glass inlet and outlet tubes. These bottles were then evacuated, the vacuum released with oxygen-free carbon dioxide and stored in the dark at – 10° F.

Preparation of tomatoes

Jones and Rosa (**7**) state that the presence and distribution of pigments varies in different varieties of ripe tomatoes as follows: In lemon-yellow fruit carotin occurs in the pericarp, but the epidermis is colorless; in orange-colored fruit, carotin occurs both in the pericarp and epidermis; in pink fruit, lycopersicin occurs in the pericarp but the epidermis is colorless; in the red fruit lycopersicin occurs in the pericarp and carotin in the epidermis and probably in the pericarp as well. Albino or white fruits lack all pigments.

The varieties of tomatoes grown were Clark's Albino, having medium sized fruit with pale yellow flesh and colorless skin; Ruby Gold, large fruit, deep yellow flesh with the locules outlined by deep red flesh, colorless skin; Globe, pink fruit with red flesh and pale yellow skin; California Earliana, medium red flesh and orange-yellow skin and Gigante Ingregnoli having small fruit of very deep red flesh and orange-yellow skin. The plants were grown at Berkeley, California, handled in a commercial manner and pruned to one stem.

The flowers were hand pollinated and bagged immediately in the same manner as the tree fruits were handled. The fruit samples also were prepared and stored in the same manner as the tree fruits.

Determination of pigments

As only relatively small quantities of fruits were available for analysis, a method of pigment extraction from wet samples was devised. Samples varying from 10 to 100 grams were analyzed.

Preliminary extraction experiments with the Schertz (**13, 14**) modification of Willstätter's method for carotin and xanthophyll, the Willstätter and Escher (**19**) method for lycopersicin and the Lubimenko (**9**)

alcohol extraction method, were unsatisfactory because of the large quantity of fruit needed, and the loss in pigments due to the many extractions and drying. At the suggestion of Dr. J. H. C. SMITH, of the Coastal Laboratories of the Carnegie Institution of Washington, who had found pyridine (b. p. 112–115° C.) a good solvent for carotin, a method was devised using this solvent. It was possible by this method to use minimum composite samples of 10 grams. Extraction with pyridine should be performed under a well ventilated hood because of the poisonous fumes. Since there was no known method of separating lycopersicin and carotin quantitatively, except by fractional crystallization, these isomers were necessarily determined together as the petroleum ether soluble pigments. Xanthophyll was determined by treating the petroleum ether solution with 80 per cent. methyl alcohol. This pigment was present only in the green fruits in concentrations great enough to give any perceptible coloration. The method of SCHERTZ (**15**) for the extraction and separation of chlorophyll, carotin and xanthophyll, was used to obtain the carotinoids from the green fruit. The depth of color in the petroleum ether solution of the carotinoids was determined with a Bausch and Lomb colorimeter of the Duboscq type.

The description of the new method used for carotin and lycopersicin extraction by the use of pyridine is given in detail.

A weighed sample of the material was triturated with washed and ignited sand and transferred to an Erlenmeyer flask. Pyridine (b. p. 112–115° C.) was added in 50-cc. portions, the first portion allowed to remain in contact with the material for 30 minutes, then decanted to a separatory funnel through a glass wool filter, retaining as much of the pulp as possible in the flask. Pyridine in amounts equal to about 15 times the weight of the sample was sufficient to remove all of the pigment. To the pyridine extractions in the separatory funnel were added equal volumes of petroleum ether (b. p. 40–60° C.) and acidulated water (equivalent to approximately normal sulphuric acid). The solution should stand from 5 to 40 minutes for complete separation of the two layers. Pyridine is miscible with water in all proportions and also forms a salt with the acid which facilitates its removal. About five washings with the acid are necessary to remove the pyridine completely. The petroleum ether layer then containing the carotinoid pigments was washed with distilled water to remove all the acid, or to the point when the liquid showed no further reaction to litmus paper. The petroleum ether solution was then filtered through a layer of anhydrous sodium sulphate and made up to a volume which produced a color nearly the same as that of the standard. Glass stoppered graduate cylinders were used for this purpose.

WILLSTÄTTER's 0.2 per cent. potassium dichromate solution equal to 0.0268 per cent. carotin, and the carotin color standard of SPRAGUE (**16**) were adopted as the carotin color standards. The use of both these standards was found necessary, the latter matching the color of the petroleum ether solutions obtained from the tree fruits and the former matching the petroleum ether solutions obtained from the tomatoes.

The pyridine removed the lycopersicin from the tomatoes along with the carotin and xanthophyll, giving a petroleum ether solution which presumably contained all the pigments. It was observed that during these extractions the pyridine removed the yellow pigments first; on further addition of pyridine the red pigment was removed, giving a deep orange-red color to the extracts. No further attempt was made to identify the pigments in the first and the last extractions by pyridine, but this appeared to be a possible means of separating these pigments without resorting to fractional crystallization. JONES and ROSA (**7**) and HOWARD (**5**) mention only two pigments present in tomatoes, the red lycopersicin and the yellow carotin. Since the lycopersicin and carotin in the petroleum ether solution were not separated, the results are expressed as milligrams of carotinoid per 1,000 grams of fruit on the assumption that the two isomers may exert the same effect.

It was necessary to discontinue the use of the pyridine method because of the poisonous effects of the fumes. The remaining fruit was analyzed by a modification of the LUBIMENKO extraction method. This method consisted of extracting the pigments from the finely ground pulp with hot 95 per cent. ethyl alcohol; the alcoholic extractions in a separatory funnel were cooled and petroleum ether added in 50-cc. portions. The carotinoids were obtained in the petroleum ether layer by diluting the alcoholic extractions with water. The combined petroleum ether extracts were filtered through anhydrous sodium sulphate and the depth of color determined as in the pyridine method. These two methods agree within 0.001 mg. per gram of fruit using the WILLSTÄTTER standard.

The moisture content of the fruit samples was determined by drying to constant weight *in vacuo* at 58° C. The pH of the fruit juices was obtained by the HILDEBRAND electrometric set up as described by CLARK (**1**).

Results

TREE FRUITS

None of the bagged fruits developed the characteristic blush on the cheek. The skin was thinner and slipped more easily from the flesh, and the Elberta and Mayflower peaches were almost entirely devoid of the characteristic fuzz. The flesh was firm, separated easily from the pit and

had the same flavor as the unbagged fruit. No difference in size of the bagged and unbagged fruits was noticeable. On removal from cold storage, all the fruits were in good condition, but on exposure to air the bagged white peaches did not oxidize as rapidly as the unbagged. This difference was observed in all other cases of bagged fruits, but none remained unoxidized in the air as long as the bagged white peaches. Table I gives the results of the determinations of moisture, pH and carotinoid content of the fruits.

TABLE I

MOISTURE, pH AND CAROTINOID CONTENT OF THE TREE FRUITS

FRUIT AND VARIETY	COLOR OF FLESH	LIGHT CONDITIONS	MOISTURE	pH	CAROTINOID PER 1,000 GM. FRUIT
			per cent.		*mg.*
Peach, Elberta	yellow	bagged 3/31/28	.....	4.31	11.0*
" "	"	bagged 6/9/28	85.6	.....	8.2
" "	"	unbagged	85.8	.....	1.9
" Mayflower	white	bagged 3/31/28	.....	4.50	no color
" "	"	unbagged	.....	4.39	no color
Nectarine, Humboldt	yellow	bagged 3/31/28	83.3	4.02	5.9
" "	"	unbagged	87.3	4.22	7.2
Nectarine, Stanwick	white	bagged 3/31/28	.....	4.51	no color
" "	"	bagged 5/10/28	82.9	4.06	no color
" "	"	bagged 6/9/28	80.4	4.14	no color
" "	"	unbagged	79.7	4.17	no color
Apricot, Royal	yellow	bagged 3/31/28	85.9	4.17	8.4
" "	"	unbagged	82.2	4.13	21.7

* These figures are averages of from 2 to 10 separate determinations.

The petroleum ether soluble pigments are calculated as carotinoids although in no case was xanthophyll found in appreciable quantities. The absence of light appears to favor the development or maintenance of the

carotinoids in the yellow Elberta peach as the bagged fruits have higher carotinoid content than the unbagged and the earlier they are bagged the higher the content of these pigments. This is not true with the other yellow fruits (Humboldt nectarine and Royal apricot) since the absence of light decreases the content of the fat soluble pigments. The carotinoid content of both bagged and unbagged fruits of the white fleshed nectarine and peach was not great enough to be measured by the method used.

GUTHRIE (**4**) found that with tomato and soybean plants grown in the dark several days, the chlorophyll content of the leaves decreased but the carotinoids remained the same. He found also a large increase in the carotin-xanthophyll ratio, which appeared to be due to a decrease in xanthophyll and an increase in carotin. If there is a change of xanthophyll to carotin this may affect the colorimetric readings in which all of the carotinoids are measured as a group and not individually. GUTHRIE also showed that reducing the light intensity to 12 per cent. of normal sunlight resulted in an increase in chlorophyll and carotinoids.

Perhaps the factors which govern the carotinoid formation or destruction in one variety of fruit are more readily catalyzed or depressed by light changes than in the others. In some varieties of fruits, light or certain wave lengths of light, may exert a destructive influence on the carotinoids during their formation or destroy them after they are formed. The exclusion of these light waves by bagging may therefore result in a higher carotinoid content, as was found in the Elberta peach. It was also shown that in some of the fruits, especially the peaches, the bagged fruits when exposed to the air, oxidized much more slowly than the unbagged fruits. If this oxidation destroys some of the pigment, bagging the fruits would result in a higher carotinoid content, other factors being equal.

TOMATO FRUITS

No differences were detected in the flavor or texture of the bagged and unbagged fruits. The mature bagged fruit was more evenly colored than the unbagged fruit. During the ripening, there was no evidence of chlorophyll formation, the fruit being pure white and gradually shading into the yellow or red as it approached maturity, indicating that chlorophyll formation in the fruit is not necessary for the normal coloring of tomato fruits. KRAUS (**8**), who was the first to work along this line, sought to show that the yellow color of the tomato and the rose was originally green, and that the yellow color originated from the green, also that the green chlorophyll-containing bodies, with or without a continuation of form, acquired a yellow color. The TOBLER'S (**17**), working with the fruit of *Momordica balsamina*, decided that throughout the ripening process the formation of carotin was

connected with the decomposition of chlorophyll. LUBIMENKO (**9**) believed that lycopersicin appears after the decomposition of the chlorophyll. He states that lycopersicin does not exist within the chloroleucites of the fruit before the decomposition of chlorophyll. Later (**10**) the same author found during the conversion of chloroplasts into chromoplasts, that chlorophyll and the accompanying pigments were decomposed, yellow pigments accumulated and then underwent alterations. He maintained that light was no longer necessary for the formation of lycopersicin after the chlorophyll had been formed. The present work shows that both light and chlorophyll, directly are unnecessary for the formation of lycopersicin in the fruit.

DUGGAR (**2**) found that the development of lycopersicin in the tomato is preceded by the paling and ultimate disappearance of the chlorophyll and that a yellowish or orange cast followed in the chloroplastids. Mature bagged fruit of Clark's Albino was almost white with a faint tinge of yellow instead of the deeper yellow of the unbagged fruit while the deposits of color of Ruby Gold were a deep red instead of the deep yellow of the unbagged fruit, indicating a probable greater increase of lycopersicin than of carotin. There was no apparent difference in the color of the mature bagged and unbagged fruit of the red fleshed varieties although the latter passed through the chlorophyll stage while the former remained white in the immature stage. Table II shows the results of the determinations of moisture, pH and carotinoid content of the fruits.

The five varieties used in these analyses can be separated into two groups with respect to their carotinoid content accompanying changes in exposure to light. Clark's Albino, having pale yellow flesh and Ruby Gold, with deep yellow flesh, show an increase in carotinoid content of the bagged over the unbagged fruits. Both of these varieties have colorless skins. Especially with Clark's Albino it appears that light may exert a destructive influence on the pigment content of the fruits, the mature green and ethylene ripened (in the dark) fruits having a greater carotinoid content than those exposed to light until maturity. WIESNER (**18**) observed that potato sprouts which formed in the light showed little if any yellow pigment while those formed in the dark developed from 30 to 150 per cent. more pigment. ELFVING (**3**) and IMMENDORFF (**6**) found that carotinoids increased greatly in leaves under conditions which depressed chlorophyll formation, that is, low temperature and very diffuse light. The fruits of Clark's Albino grown in the greenhouse, resulting in reduced light and with the shorter wave lengths removed, also had higher content of the carotinoids than those grown outside and exposed to the full sunlight. In the second group, composed of Globe, having red flesh, California Earliana, of medium red flesh and Gigante Ingregnoli with deep red flesh the unbagged fruits, both outdoor and greenhouse grown, have a higher carotinoid

TABLE II

MOISTURE, pH AND CAROTINOID CONTENT OF TOMATO FRUITS

VARIETY AND NORMAL COLOR	TREATMENT	MOISTURE	pH	CAROTINOID PER 1,000 GRAMS OF FRUIT	COLOR STANDARD USED
		per cent.		*mg.*	
Clark's Albino	Outdoor grown, mature green	92.4	4.43	0.23*	Sprague
Pale yellow flesh	" " ripe unbagged	93.6	4.27	no color	———
Colorless skin	" " ripe bagged	91.7	—	too dilute to read	———
	" " ethylene ripened	93.6	4.39	0.56	Sprague
	Greenhouse grown, unbagged ripe	91.8	4.19	0.29	"
	" " ripe bagged	—	4.17	2.50	"
Ruby Gold	Outdoor grown, ripe unbagged	92.5	4.43	3.40	"
Deep yellow flesh	Greenhouse grown, ripe unbagged	92.1	3.72	0.30	Willstätter
Colorless skin	" " " bagged	88.5	4.18	3.25	"
Globe	Outdoor grown, ripe unbagged	90.9	4.23	3.45	"
Red flesh	" " " bagged	91.5	4.27	2.50	"
Pale yellow skin	Greenhouse grown, ripe unbagged	88.7	4.07	3.45	"
	" " ripe bagged	90.4	4.18	1.75	"
	" " mature green	89.8	4.31	1.10	Sprague
	" " one-half ripe	—	4.00	1.40	Willstätter
	" " three-fourths ripe	—	4.23	1.40	"
California Earliana	Outdoor grown, ripe unbagged	90.8	4.24	5.30	"
Medium red flesh	Greenhouse grown, ripe unbagged	90.2	4.30	2.15	"
Orange yellow skin					
Gigante Ingregnoli	Outdoor grown, mature green	91.8	4.26	3.20	Sprague
Deep red flesh	" " ripe unbagged	91.8	4.26	3.05	Willstätter
Orange yellow skin	" " " bagged	90.8	4.38	1.80	"
	" " ethylene ripened	91.2	4.19	6.30	"
	Greenhouse grown, ripe unbagged	90.6	4.15	5.15	"
	" " ripe bagged	90.9	4.49	1.40	"

* These figures are averages of from 2 to 10 separate determinations.

content than the bagged fruits, suggesting that light is directly related to carotinoid development or preservation in these varieties. The varieties of this group have colored skins although that of the Globe variety is very pale yellow and often classified as colorless.

The fruit of the Globe variety analyzed at the various stages of immaturity had low carotinoid content. In only one case (Gigante Ingregnoli) was the carotinoid content of greenhouse grown fruits higher than those grown outside. The differences in the hydrogen-ion concentrations of bagged and unbagged fruits also should receive consideration. With one exception, Clark's Albino, greenhouse grown, where there is but 0.02 pH difference, the bagged fruits have a higher pH than the unbagged ones. It appears highly probable that these changes in hydrogen-ion activity may affect the enzyme activity and other metabolic action of the fruit which would alter their pigment content. The differences in carotinoid content between greenhouse and outdoor fruits grown at Berkeley, where very little ultraviolet rays reach the outdoor grown plants, may be altered if grown where more of these rays reached the plants. However, light on the fruit is unnecessary for the production of carotinoids in those fruits.

DUGGAR (**2**), using green fruits which had been exposed to light until almost ready to turn color, showed that light was unnecessary for the normal pigmentation of red tomatoes.

It is possible that part of the variation in the pigment content reading was due to the changing relative amounts of carotin and lycopersicin present which were extracted and determined unseparated. WILLSTÄTTER and ESCHER (**19**) and MONTEVERDE and LUBIMENKO (**11**) have shown that the principal pigment in the tomato is lycopersicin although carotin is present in small amounts.

Summary

1. A new method is described for the extraction of the carotinoid pigments from fresh tissue using pyridine (b. p. 112–115° C.) as the solvent.

2. Peaches, apricots and nectarines bagged in the blossom stage and developing in total darkness do not oxidize as rapidly when exposed to the air as those developing in full light.

3. The bagged Elberta peach developed a higher carotinoid content than the unbagged while the reverse was true of the Humboldt nectarine and Royal apricot. The Mayflower, white fleshed peach, and Stanwick, white fleshed nectarine, contained such small quantities of the carotinoids that no quantitative determination could be made.

4. During the ripening of tomato fruits in total darkness no chlorophyll developed. The fruits were pure white and gradually shaded into the yellow or red as they approached maturity. When mature, the bagged and unbagged fruits of the red varieties were the same color.

5. Mature bagged fruit of Clark's Albino was almost white with a faint tinge of yellow instead of the deeper yellow of the unbagged fruit. The localized deposits of color of Ruby Gold were a deep red instead of the deep yellow of the unbagged fruit.

6. Fruits of Clark's Albino and Ruby Gold, having pale yellow and deep yellow flesh, respectively, show an increase in carotinoid content of the bagged over the unbagged. Fruits of Clark's Albino grown in the greenhouse also had a higher content of carotinoids than those grown outside.

7. Both the outdoor and the greenhouse grown fruits of the three red fleshed varieties had a lower carotinoid content when bagged.

8. With one exception, greenhouse grown Clark's Albino where there was but 0.02 pH difference, the bagged fruit had a higher pH than the unbagged fruit.

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STUDIES ON INANITION IN *ARACHIS* AND *PHASEOLUS*

G. M. Shear

(WITH FOUR FIGURES)

I. Introduction and literature

The changes that occur in organisms during starvation or inanition have been the subject of many investigations by animal and human physiologists, but very little has been done with plants, and the botanical literature on this subject is consequently very meager. Even workers on the animal side, Jackson (7) and Howe (5), have noted this and have expressed the desirability of a more thorough understanding of the effects of inanition on plants. It is only through studies of many organisms under varying conditions that we may hope to get at the fundamental likenesses and differences in the metabolism of plants and animals. The purpose of this paper is to add to our knowledge of the changes that take place in plants during inanition, induced by the checking and removal of the food supply stored in the cotyledons.

Inanition in its broadest sense refers to the subsistence of an organism on one or more of the essential mineral or organic substances contained within its body. Morgulis (13) divides inanition into three main types, physiological, pathological, and experimental. The first refers to a normal condition in the life cycle of an organism, such as hibernation in animals. The second is the result of an organic derangement produced by some disease. The last is the withholding of nutriment, generally for the purpose of scientific studies. The latter two produce very marked changes from the normal functioning of the organism, the extent of these depending on the severity of the inanition. Jackson (7), on the other hand, divides inanition into total or quantitative and partial or qualitative, each of these groups being, in turn, divided into complete and incomplete inanition. He considers plants growing in distilled water in the absence of light as being subjected to conditions of total inanition.

Starvation considered from the most widely accepted standpoint consists merely of the withholding of organic matter from an organism and the author uses it in that sense. The availability of inorganic salts is of little or no direct consequence in prolonging life in the absence of food. Therefore unless an organism has an excessive amount of food stored up, the salts which it contains will be sufficient to more than supply its needs until it dies from lack of food, or from a disruption of its metabolic processes due to the depletion of its food supply. From a physiological point of view however, an organism is not actually starving until its food supply

has been depleted to an extent that causes a derangement of its normal metabolic functions. A seedling growing in the dark in the absence of extraneous organic material grows normally until it is well above the medium in which it is planted. PEARL, WINSOR, and MINER (**16**) found that the rate of increase in the length of shoots of muskmelon seedlings grown in the dark on agar at 30° C. was very rapid for about ten days. This was followed by a rapid falling off lasting about two days, after which elongation ceased. Starvation as effecting shoot elongation made its first appearance in approximately ten days. These first stages of growth in seedlings might be classified under the heading of physiological inanition as given by MORGULIS (**13**).

In discussing fasting HOWE (**5**) says that the fat available in an organism exerts a marked effect upon the metabolism. "So long as there is sufficient fat in the organism to supply the energy requirements, the protein metabolism will remain at a minimum. When, however, the fat deposits are depleted, the body is forced to use protein to furnish the necessary energy. The result is a more rapid protein consumption and an early death."

According to JACKSON (**6**), plants, much more than animals, appear susceptible to modification by various external factors including the food supply. Sooner or later during inanition in all living organisms the cells pass from a stage of simple atrophy into a condition of degeneration. The recovery is thus seen to be dependent upon the progress of inanition.

HOTTES (**4**) in his cytological studies of starving seedlings of the broad bean, *Vicia faba*, found that there was an increase in vacuolization and that the cytoplasm became so attenuated that it was barely visible as a narrow band in close contact with the cell wall. The quantity of chromatin also was reduced. He found that the primordia of the secondary roots ceased development, as the food is utilized by the active cells at the tip of the main root.

COUPIN (**2**) found that seedlings grown in distilled water in darkness lived various lengths of time. He reports that seedlings of the nut pine grown in this way lived 60 days; those of pumpkin 46 days; of winter vetch, 44 days; of lentil, 40 days; of marvel of Peru, 39 days; of pea, 33 days; of bean, 32 days; of sunflower, 30 days; of buckwheat, 25 days; of radish, 24 days; of nasturtium, 23 days; of spinach, 22 days; of tomato, 21 days; of beet, 20 days; of common cress, and mustard, 18 days. He attributes this "resistance to inanition" as being due especially to the quantity and quality of the reserve material in the seed.

BOUYGUES (**1**) and VON PORTHEIM (**17**) have studied certain phases of starvation in the seedlings of *Phaseolus vulgaris*. Both workers found that seedlings without cotyledons developed into much smaller and weaker plants

than those with cotyledons. The former claims that there is a balance maintained between the root and shoot of the check and starving seedlings. The latter found that the length and number of internodes developing corresponds to the reserve material, originally at the disposal of the seedlings. He noted a growth acceleration in the hypocotyl of seedlings with one or part of both cotyledons removed, which he attributed not to a stimulation from wounding, but to nutritional disturbances brought about by the lowering of the amount of reserve food. This phenomenon has been given the name hunger etiolation by NOLL (**14**).

II. Materials and methods

Seedlings and embryos of the Spanish peanut, *Arachis hypogaea,* and seedlings of the common garden bean, *Phaseolus vulgaris,* were used.

Seeds weighing more than 0.550 grams were treated for forty-five minutes with a 0.025 per cent. solution of Uspulun, and from them the cotyledons were removed in a steamed cabinet to insure sterile conditions. The embryos were placed in test tubes containing approximately 15 cc. of 1.5 per cent. sterile agar. The tubes were kept in a dark room at an average temperature of 22° C. At two day intervals sets of four embryos were placed five feet beneath an 1834 candle power light to test for chlorophyll formation. The intensity of the chlorophyll was determined at the end of the first and second days of exposure, by a comparison with RIDGEWAY'S color charts (**20**).

The seeds used in the growing of seedlings were selected so as to fall within five per cent. of the average air dry weight of a random sample of seeds. This was 0.500 to 0.550 grams for peanuts and 0.400 to 0.440 grams for beans. These seeds were kept in a covered can in a desiccator without a drying agent so as to maintain the original moisture content. Samples of these seeds were dried and the moisture content and seed-coat weight determined. The average of these two was for peanuts 33 ± 0.435 and for beans 74 ± 0.672 mg. From these the calculated dry weight and seed-coat weight of each individual seed was determined.

Seedlings for the determination of chlorophyll formation were grown in glass jars in pure silica sand, six seedlings to a jar. These were grown at the same temperature as the embryos. The cotyledons were removed from half of the seedlings after six days. At two day intervals until chlorophyll ceased to form in the starving seedlings, one jar of each with a check jar was placed beneath an 1834 candle power light. The tops of the seedlings were approximately four feet from the light. Determinations of the chlorophyll intensity were made at the end of one and two days, as with the embryos. The seedlings being susceptible to injury from excessive transpiration had to be kept at a humidity above that of the room. This

was accomplished by placing the jars in which the seedlings were growing in a tub, the bottom of which was kept covered with water, and over this another tub was inverted. The jars of plants under the light were also placed in a tub containing water.

In the experiments to determine loss of weight during inanition, weight records of individual seeds were kept. The seedlings were grown in glass jars filled with pure silica sand containing 50 per cent. of its water holding capacity. Eight jars containing a total of thirty seeds constituted a set and were grown in a wash boiler with a ventilated top. All sets were grown at 23° C. and the cotyledons were removed from half at the end of six days. They were then grown for different lengths of time, some sets at 23° C., others at 30° C.

To prevent undue oxidation of the peanut oil at high temperatures, all of the drying was done in a vacuum oven at 75° C. until a constant weight was reached.

Some difficulty was encountered in securing the dry weight of roots of seedlings grown in quartz sand. It was found impossible to remove all of the sand from the roots by merely washing them. It was therefore necessary to get the weight including the sand which adhered to them, then burn the material, wash off the ash, and get the weight of the sand. In many instances the sand weighed more than the roots. Some investigators have ignored the sand that adheres after washing and consequently have introduced a big source of error.

Ether extract determinations by means of a Soxhlet extractor were made on starving and check seedlings of the peanut. These were grown at 23° C. as in the preceding experiments and determinations made on groups of ten plants at two day intervals for a period of from six to eighteen days. The seedlings were dried, ground in a mortar, and then dried again for several hours before weighing. Porous bottomed crucibles were used to hold the material, instead of paper extraction thimbles as they are much easier to handle and the chances for error in weighing are less. After extraction for twelve hours the material was dried and reweighed.

III. Results and discussion

Chlorophyll formation and growth of peanut embryos

It is a well-known fact that many plant embryos have enough inherent vitality to grow and form chlorophyll. The length of time that this will occur after growing in the dark, and the intensity of the greening, are indications of physiological readjustments to meet the demands of growth and maintenance.

From the twelfth day of growth through the twentieth day there is a gradual decrease in the intensity of the greening characterized by changes

TABLE I

INTENSITY OF CHLOROPHYLL IN PEANUT EMBRYOS

GROWTH PERIOD	TIME IN LIGHT	COLOR
days	*hours*	
10	24	Scheele's green
	48	Scheele's green
12–14	24	Green-yellow
	48	Scheele's green
16	24	Viridine green
	48	Scheele's green
18	24	Viridine green
	48	1 Scheele's green 3 Viridine green
20	24	Light viridine green
	48	Viridine green
22–35	24	Pale cendre green
	48	Viridine green
38	24	Pale cendre green 1 no chlorophyll
	48	Viridine green 1 no chlorophyll

in both hue and tone (table I). The hue becomes greener and the tone lighter. A gradual decrease in the intensity of the yellow could be noted at the time the embryos were placed in the light. This decrease in yellow pigment, probably carotin, was responsible for the change in hue. From the twentieth to the thirty-eighth day at which time the embryos start losing their ability to form chlorophyll the rate of greening remains constant. At first the epicotyl and leaflets turn green, but there is a gradual decrease in the area of greening in the leaflets from their tip to base, and in the final stages of chlorophyll formation only the epicotyl and base of the leaflets turn green.

The embryos practically cease elongation after four weeks. Measurements taken on twenty-six of them after thirty days gave an average shoot length of 2.0 cm. and a root length of 5.9 cm. The shoot length represents mostly hypocotyl as the epicotyl is not more than 1 or 2 mm. in length. Most of the embryos produce one to several secondary roots which show their great vigor. As shown by HOTTES in *Vicia faba* and by some preliminary work of the author with beans, peas, pumpkin, and squash, the removal of the cotyledons during germination and before the secondary

root primordia have reached too advanced a stage, inhibits their further development.

The embryos with excised cotyledons grown to find out how long they were capable of living, probably died from desiccation rather than inanition. One, however, lived for one hundred and forty days before the agar dried up. This, when compared with the length of life, (twenty some days), of peanut seedlings whose cotyledons were removed after six days' growth, shows that the rate of metabolism at the time that starvation is induced has a marked effect on the length of life. The embryos are able to utilize their small reserve of food much more economically than seedlings with a much greater reserve. From these results it would seem that HOWE's (5) statement, "adult organisms can fast longer than the young of the same species" does not necessarily hold for plants.

CHLOROPHYLL FORMATION IN SEEDLINGS OF THE PEANUT AND BEAN

The intensity of greening at different periods of growth is shown in table II. As in the embryos there is a decrease in the amount of yellow pigment in the starving seedlings. After seventeen days in the case of the beans and nineteen for peanuts with the cotyledons removed, chlorophyll is no longer formed or at least not in quantities large enough to be detected by the method used. With the advance of inanition it is always the young-

TABLE II

INTENSITY OF CHLOROPHYLL FORMATION IN SEEDLINGS

GROWTH PERIOD	LIGHT	COLOR, COTYLEDONS REMOVED	COLOR, CHECK
		PEANUTS	
days	*hours*		
15	24	Viridine green	Scheele's green
	48	Scheele's green	Cossack green
17	24	Light yellow-green	Scheele's green
	48	Green-yellow	Grass green
19	24	Pale greenish-yellow	Scheele's green
	48	Pale greenish-yellow	Grass green
	72	Pale greenish-yellow	Grass green
		BEANS	
15	24	Light yellow-green	Yellow-green
	48	Colliste green	Scheele's green
17	24	Light greenish-yellow	Green-yellow
	48	Light greenish-yellow	Scheele's green
19	24	Light greenish-yellow	Scheele's green
	48	Light greenish-yellow	Scheele's green
	72	Light greenish-yellow	Scheele's green

est leaves that show the most intense greening, and that retain the power of forming chlorophyll the longest. The older leaves gradually cease forming chlorophyll from their outer edges toward the base. In order to obtain as much uniformity as possible all color determinations were made from the youngest leaves.

As PALLADIN (**15**) states one of the prerequisites for chlorophyll formation is the presence of soluble carbohydrates. The ability of the seedlings to form chlorophyll and the amount of chlorophyll formed are undoubtedly an index of the available carbohydrates and therefore indicate the progress of starvation. This is substantiated by the fact that the older leaves failed to form chlorophyll while the younger ones were still capable of doing so. The soluble carbohydrates would be used up in the older leaves or transported to the younger ones, thus depleting their supply first.

GROWTH OF PEANUT AND BEAN SEEDLINGS

As stated in the methods, thirty seeds were planted in each set, half of them to be used as checks. This was done to assure at least ten plants for each group. Little difficulty was experienced with peanuts as can be seen from the number of seedlings in each group. Most of the loss came through lack of germination. The greatest difficulty experienced with beans was the tendency for them to come up with one or both cotyledons broken off. The main root in some of the beans could not free itself of the seed-coat, thus making the seedlings worthless. A few seedlings because of their abnormal growth were discarded.

Six days after planting was selected as the time for the removal of the cotyledons from the seedlings to be starved, since at that time the cotyledons of the peanuts were just above the surface of the sand. The dry weights obtained from the starving plants show that there is considerable variation in the actual weight of the seedlings from different sets at the time of cotyledon removal. This makes direct comparisons of different sets difficult in some cases although the relationships expressed in percentages negates such variations to a great extent.

Beans elongate more rapidly than peanuts during the first six days of growth (tables III and IV), although the average dry weight of the seedlings is practically the same. This means that the peanuts are much more stocky than the beans. This stockiness is due to the thick cortex of the hypocotyl. WALDRON (**21**) in his work on the peanut says that the hypocotyl often becomes thick and fleshy in its cortex. "This is more marked when growth is retarded from some cause and then the lower end becomes tuberous from a deposition of sugar. The roots of such are not able to utilize the food as fast as it is supplied from the seed." That the cortex of the hypocotyl is a reservoir of available food for the seedling is clearly

shown in fig. 2 by the wrinkled and withered condition of this region after the food has been utilized during inanition. This wrinkling occurs progressively from the upper portion of the hypocotyl toward the base.

The secondary roots of the peanut were less than a centimeter long at the time of cotyledon removal, whereas, in the bean there were usually four secondaries that were several centimeters long. During the course of inanition the secondaries of the peanut increased in number and the first ones reached a length of from 2 to 3 centimeters, (fig. 1). In the bean the four original secondaries more than doubled in length while none of the others developed far beyond the primordial stage.

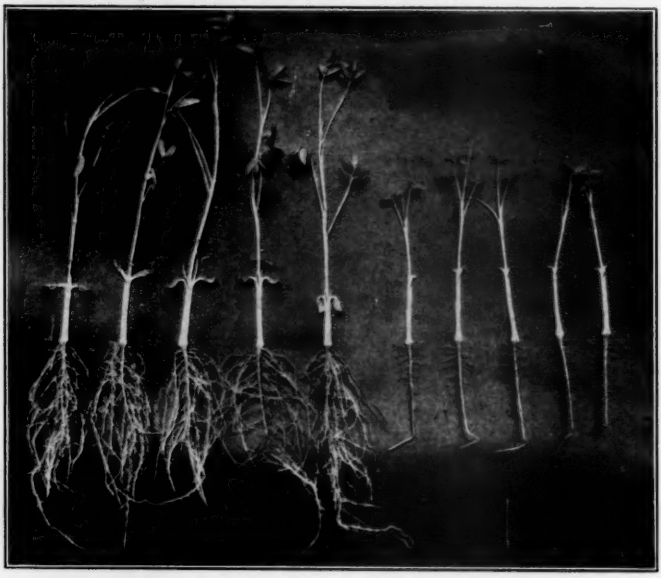

FIG. 1. Peanut seedlings after eighteen days growth at 23° C. Check plants at the left, at the right plants from which the cotyledons were removed after six days.

The general appearance of the starving plants was found to agree with the observations of VON PORTHEIM (**17**) and BOUYGUES (**1**). They were smaller, developed fewer and shorter internodes, and produced fewer and smaller leaves. The hypocotyls of the starving peanuts were slightly longer than were those of the checks (fig. 2). VON PORTHEIM found this to be true of bean seedlings with one or parts of two cotyledons removed, although, as confirmed by the author, beans with both cotyledons removed did not show this phenomenon.

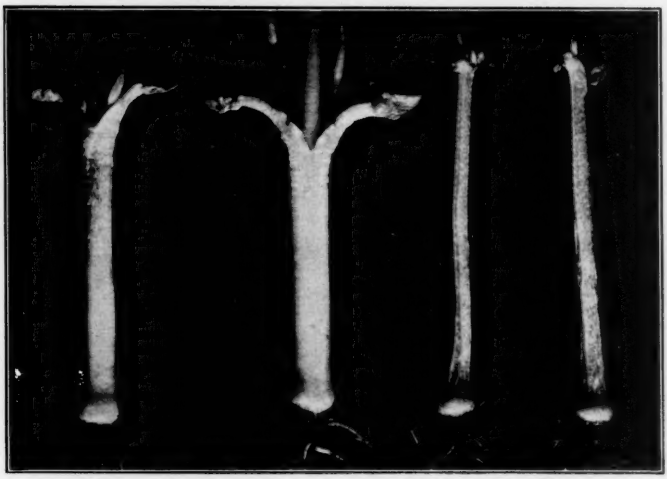

FIG. 2. A detailed view of the hypocotyls of four of the seedlings shown in figure 1. Note the wrinkling and etiolation of the starving seedlings at the right.

The ability to adjust themselves to abnormal conditions seemed to be greater in the starving peanuts than in the checks. The check seedlings growing at 23° C. began developing translucent (watery) areas around the outer edge of the oldest leaves after fourteen days. These areas continued to enlarge and were apparently due to a "water logged" condition of the tissues. Upon exposing such plants to the dryer air of the laboratory these areas dried and turned brown, showing that the cells in such areas were dead. The starving seedlings failed to show this injury and were in good condition upon removal. Peanuts grown eight days at 30° C., after the removal of the cotyledons from half of the plants, showed a different type of injury. On the fifth day at 30° C. the terminal buds of all but two of the checks were dead and appeared black, whereas the starving plants showed no injury. At the time of removal of the set the terminal buds of the starving seedlings appeared straw colored and watery.

With regard to elongation in the peanuts, the roots seem to be the first to be affected by inanition. Even on the tenth day they were much shorter than those of the checks (table III). The shoots elongate at approximately the same rate through the tenth day, after which the checks greatly outclass the experimental plants.

The increase in seedling weight of the checks, (cotyledons excluded), was very rapid through the fourteenth day, after which the rate of transfer of food from the cotyledons was practically balanced by the loss through

TABLE III

GROWTH OF PEANUT SEEDLINGS

No. of plants	Growth period	Starved	Average calculated dry weight of seed minus seedcoat	Average dry weight of cotyledons	Average dry weight of shoot	Average dry weight of root	Total weight of seedlings	Average loss in weight	Average length of shoot	Average length of root	Average total length
	days	*days*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*cm.*	*cm.*	*cm.*
A. At 23° C.											
12	6	check	0.496	0.386	0.085	0.021	0.106	0.003	3.6	8.5	12.0
12	10	check	0.495	0.219	0.214	0.061	0.276	0.000	10.9	16.0	26.9
12	10	4	0.494	0.367	0.086	0.023	0.109	0.016	10.0	10.7	20.7
12	14	check	0.498	0.086	0.275	0.095	0.368	0.042	22.0	21.4	43.4
12	14	8	0.494	0.362	0.085	0.023	0.107	0.024	15.1	12.1	27.2
14	18	check	0.498	0.055	0.274	0.099	0.372	0.070	28.8	22.9	51.7
12	18	12	0.502	0.348	0.093	0.028	0.122	0.032	19.0	14.8	33.8
B. At 30° C. from the sixth day of growth—											
14	10	check	0.495	0.092	0.259	0.100	0.359	0.045	19.7	23.5	43.2
12	10	4	0.491	0.354	0.086	0.024	0.110	0.026	14.6	15.1	29.7

respiration during the next four days. The cotyledons still contained a small amount of food on the eighteenth day. Due to variations in the weight at the time of cotyledon removal no direct comparisons of the starving plants can be made.

After six days' growth the peanut seedlings showed an average loss in weight, (based on the calculated dry weight of the seed minus the average seed-coat weight), of 3 mg. (table III). The loss in weight of the seedlings without cotyledons showed a steady increase, while the average loss for the checks at ten days was 0 mg. From this time on to the end of the experiment the checks showed a very rapid loss. Results for check seedlings between those of six and ten days gave an average gain of 4 mg. At this time the plants were quite large and the gain would have to be proportionally large to offset the loss through respiration. A gain in weight of seedlings from oily seeds has been recorded by several investigators. As early as 1855 HELLRIEGEL (**3**) detected an increase in the weight of rape seedlings during the early stages of germination. JEGOROW (**8**) found that the oil content and also the dry matter of *Cucurbita maxima* seedlings had increased at the end of six days' growth.

This increase is undoubtedly due to the decomposition of the oily reserve which from RHINE'S (**19**) results seems to be necessary for translocation. This entails, first, hydrolysis, and then partial oxidation. In the first of these processes water is chemically combined at the rate of three molecules for every morecule of fat. Oxygen then combines with the fatty acids to form intermediate compounds such as sugar. These processes must have produced a large excess of readily available food between the sixth and tenth days of growth.

Peanuts starved four days at 30° C. show the same general tendency as those grown at 23° C. Their growth is more rapid and consequently they lose more weight. The checks show a much greater loss than check seedlings grown for the same period of time at 23° C. Because of the injury to the seedlings at this temperature, records on longer periods of growth could not be obtained.

The length of the shoots of check beans grown at 23° C. increased rapidly until the fourteenth day, after which it practically ceased (table IV). The shoots of starving seedlings showed the same tendency. The roots, however, in both groups showed very little elongation after the tenth day. The general tendency of elongation would therefore seem to be more dependent on the age of the bean seedlings than upon their food-supply. Peanuts did not show this.

The changes in weight of the seedlings (exclusive of the cotyledons) was of necessity quite different as the checks will continue to increase as long as they can get sufficient food from the cotyledons, while the others

TABLE IV

GROWTH OF BEAN SEEDLINGS

No. of plants	Growth period	Starved	Average calculated dry weight of seed minus seedcoat	Average dry weight of cotyledons	Average dry weight of shoot	Average dry weight of root	Total weight of seedlings	Average loss in weight	Average length of shoot	Average length of root	Average total length
	days	*days*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*cm.*	*cm.*	*cm.*
A.	At 23° C.										
10	6	check	0.344	0.197	0.084	0.020	0.104	0.043	9.9	14.2	24.1
10	10	check	0.339	0.034	0.174	0.037	0.211	0.094	29.3	27.8	57.1
11	10	4	0.346	0.208	0.073	0.020	0.094	0.044	20.3	20.6	40.9
10	14	check	0.352	0.032	0.185	0.035	0.219	0.100	40.7	27.3	68.0
11	14	8	0.348	0.198	0.076	0.021	0.098	0.052	29.0	22.0	51.0
10	18	check	0.342	0.032	0.165	0.036	0.201	0.109	41.6	28.8	70.4
12	18	12	0.347	0.206	0.067	0.020	0.088	0.053	30.6	20.8	51.5
B.	At 30° C. from the sixth day of growth—										
10	10	check	0.340	0.031	0.175	0.038	0.213	0.095	32.0	27.0	59.1
11	10	4	0.345	0.165	0.099	0.024	0.123	0.057	26.0	24.5	50.5
10	14	check	0.344	0.032	0.165	0.035	0.200	0.113	42.3	27.0	69.4
9	14	8	0.345	0.179	0.081	0.023	0.103	0.062	32.1	21.1	53.2

will start losing when their supply of food is removed. The root and shoot weights of the checks was found to be proportional to their lengths. The root and shoot weights of the starving plants show that the root weight remained fairly constant, while most of the loss was from the shoot.

The loss in weight of the checks was very rapid up to the tenth day and from then on it was much slower. This decrease in the rate of loss occurs at about the same time that the reserve food has been completely removed from the cotyledons and is an indication of inanition. The loss of weight in the starving plants only averaged 10 mg. in the twelve days of inanition as compared with 66 mg. loss for the checks during the same period.

Both check and starving beans grown for six days at 23° C. and then transferred to 30° C., show a greater and more rapid shoot elongation than do those that are not transferred, but that are grown at 23° C. (table IV). They maintain the same relationship to each other regardless of their more rapid growth.

The loss in weight of peanuts during the period of induced inanition at 23° C. is approximately three times as great as for beans during the same period. The checks of both on the other hand lost practically the same amount during this time although the beans lose most rapidly from the sixth to tenth days while the peanuts lose most from the tenth day on. The peanuts in spite of the loss of their cotyledons have enough reserve food in their hypocotyls to maintain a higher rate of metabolism than beans under similar circumstances. The food from the bean cotyledons is translocated at a slower rate than it is from the cotyledons of peanuts. This accounts for the accumulation in the hypocotyl.

That starvation upsets the normal ratio of root to shoot growth is clearly shown in figure 3, where the percentages of shoot weight and length to total weight and length are given. While weight is a more accurate measure of growth than length, these show a consistent correlation with more of a tendency for the curves to converge in the length measurements. A very significant difference in the reaction of peanuts and beans to inanition is shown by these data. In the peanuts the percentage of shoot weight and length to total weight and length is less for the starving seedlings than for the checks. This relationship is just the reverse for beans. When the results of the starving beans and peanuts with regard to percentage shoot weight to total weight are compared, as is shown in the following data,

Beans	80.6	78.2	78.4	76.1
Peanuts	80.5	78.5	78.8	76.8

a remarkable uniformity is seen. It is probable that through the removal of the cotyledons the carbohydrate-nitrogen ratio is interfered with. REID (**18**) has shown, that the root-shoot ratio of different kinds of seedlings grown in the dark is more or less dependent upon their carbohydrate-

nitrogen ratio. Seedlings with a large relative amount of carbohydrates produce proportionally greater root systems. It would be interesting to determine this ratio for starving seedlings of beans and peanuts grown as described to see whether the same carbohydrate-nitrogen ratio in different plants is indicated by their having the same root-shoot ratio.

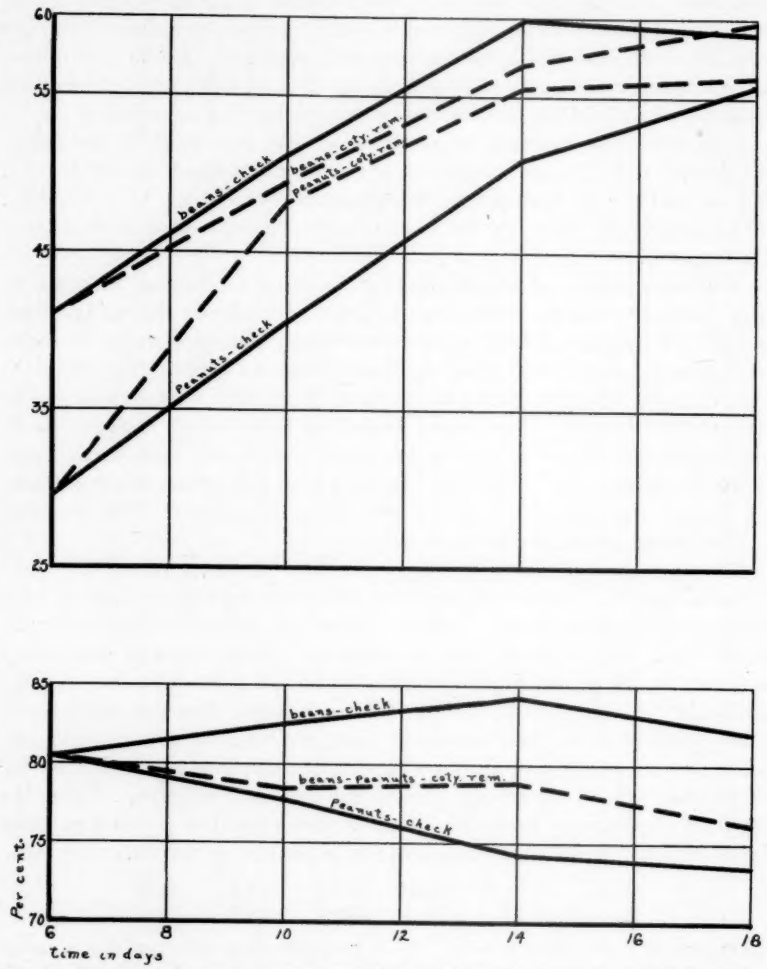

FIG. 3. Upper graph, per cent. shoot length to total length of peanut and bean seedlings grown at 23° C. Lower graph, per cent. shoot weight to total weight of peanut and bean seedlings grown at 23° C.

Oil content of peanut seedlings

All of the determinations were made after discarding the cotyledons in order to make the checks comparable to the starving plants.

The results shown in figure 4 give the per cent. of oil, as ether extract, that is found in the seedlings at two day intervals of growth from the sixth

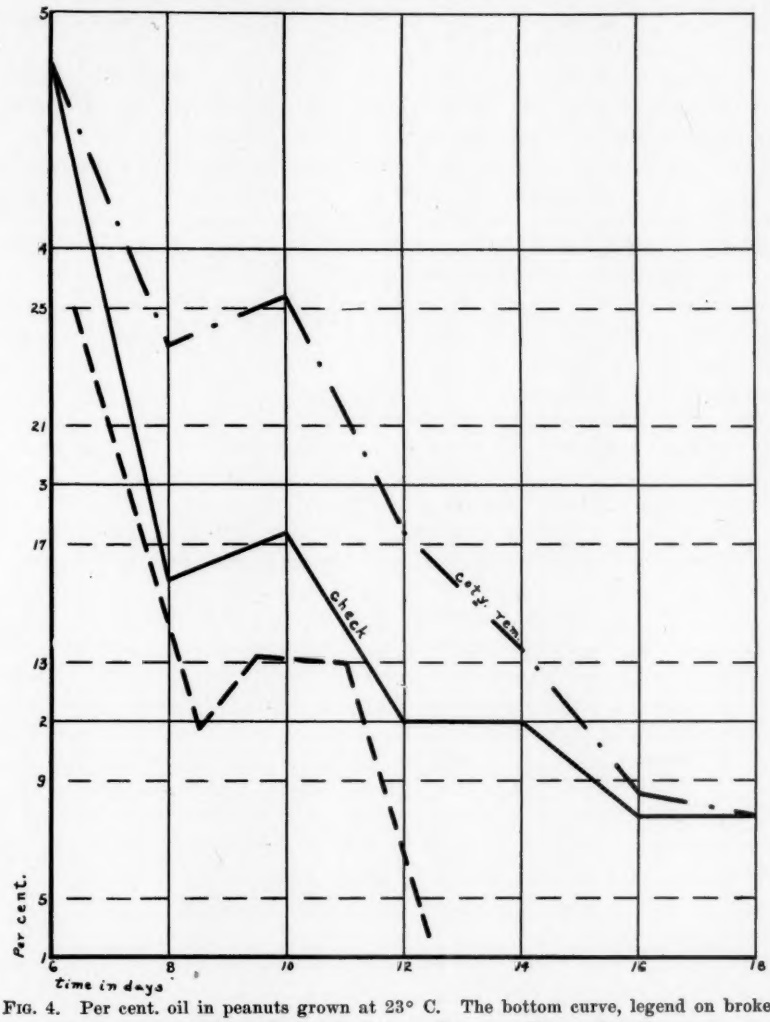

Fig. 4. Per cent. oil in peanuts grown at 23° C. The bottom curve, legend on broken lines, represents the per cent. of oil in sunflower seedlings, after Matthes.

through the eighteenth day. A small percentage of the extract probably consists of cutin, and other ether soluble substances that are unavailable as food. This may explain the presence of over 1.5 per cent. extract from the starving seedlings at eighteen days when they have nearly reached the point of death. From the sixth to the eighth day the seedlings with their cotyledons removed show a marked decrease in oil content, followed by a slight rise during the next two days, after which there is a steady decrease up to the eighteenth day. Until the eighteenth day, the loss in percentage of oil in the check seedlings is more rapid than in the experimental plants. On the eighteenth day the per cent. is the same in both groups. As shown by figure 4, the loss shows a definite four day cycle, two days of decrease followed by two days during which the percentage remains practically constant. It is probable that the graph would not have shown as much regularity if different intervals of time had been used in making determinations. Shorter intervals would undoubtedly have given a very irregular curve as not all plants reach the same stage at the same time. The works of MAQUENNE (**9**) and MILLER (**11**) show the effect of too long and irregular intervals of time upon oil determinations. MAQUENNE found the following percentages of oil in peanuts during germination: 51.39 at 0 days, 49.81 at 6 days, 36.19 at 10 days, 29.00 at 12 days, 20.45 at 18 days, and 12.16 at 28 days. MILLER obtained the following results with sunflowers: 9.9 at 3.5 days, 7.4 at 5 days, 3.8 at 7 days, 3.1 at 10 days, and 1.8 at 14 days. He (**12**) says that the per cent. of ether extract falls very rapidly, and after the cotyledons are above ground it remains almost constant.

The more recent work of MATTHES (**10**), part of which is represented in figure 4, shows that sunflowers have the same tendency as peanuts to give a decrease in the percentage of oil followed by a slight increase. It is also interesting to note that although the percentage of oil is higher in his determinations, due to his including the cotyledons, the time and intervals of fluctuation practically coincide with the results for peanuts. He also found a rather regular rise and fall in the percentage of some of the intermediate products of the decomposing oil.

These results show clearly that the rate of oil consumption which should be a good index of metabolism is markedly reduced in seedlings with their cotyledons detached. During the early stages before the inanition becomes severe the oil pattern is the same as for the seedlings with an abundant food supply. Although they are unable to maintain this uniformity, they show themselves to be more conservative in the use of the oil at their disposal.

IV. Summary and conclusions

1. Peanut embryos growing at room temperature in darkness on sterile agar are capable of living more than four months. They cease elongation

in about four weeks and lose their ability to form chlorophyll after six weeks.

2. Embryos of peanuts live longer and form chlorophyll for a longer time than seedlings of similar plants in which inanition has been induced by removal of the cotyledons after active growth has started.

3. Seedlings of peanuts whose cotyledons have been removed after six days' growth will form chlorophyll for a longer time than will seedlings of beans similarly treated. The gradual decrease in the intensity of greening is accompanied by a decrease in the amount of yellow pigment.

4. The loss in weight of peanut seedlings with their cotyledons removed is nearly three times that of beans treated in the same way. This is due chiefly to the amount of excess food translocated to the hypocotyls previous to cotyledon removal.

5. The per cent. of shoot length to total length and shoot weight to total weight in starving peanuts was found to be greater than for check plants. This relationship is just the reverse for beans. A very close correlation exists between the per cent. shoot weight to total weight of starving peanuts and beans grown at 23° C.

6. Starving seedlings of peanuts utilize their oil reserve more economically than the checks. After the first six days of inanition they were unable to maintain the regular fluctuations in the per cent. of oil that was shown by the check seedlings.

The author wishes to express his sincere appreciation to Dr. C. F. HOTTES for his suggestions and helpful criticism throughout the course of the work.

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COMPARISON OF THE CHEMICAL COMPOSITION OF SOME MARINE ALGAE

MARGARET R. BUTLER

Very few analyses of marine algae of the Atlantic coast have been reported in the literature as compared with those of the Pacific coast forms. There exist, furthermore, sharp differences in the type and abundance of species in these two regions. For Canadian waters of the Atlantic coast no analyses have yet been reported. The abundance of this material on the shores of the Maritime Provinces establishes it as a natural resource of possible importance. With this idea in mind analyses have been carried out on the more abundant forms for their content of substances of economic importance. In other words, the present report is intended as a survey as well as a comparison with the same or allied species to be found in other parts of the world.

Until displaced in 1873 by the iodides of the great salt deposits, seaweed was the world's chief source of iodine. Yet during the stress of economic conditions imposed by the world war, it was reverted to as a commercial source of iodine in the United States.

The United States Department of Agriculture also became interested in the production of potash from kelps and operated an experimental and demonstrational kelp products plant at Summerland, California, the account of which appears in "Potash, a review, estimate and forecast" (Wiley), and in a series of articles in the "Journal of Industrial and Engineering Chemistry." It is interesting as having been one of the first utilized products of seaweed.

The sale of dulce in certain localities as a human food and as a medicinal lends interest to the determinations of the protein content calculated from the nitrogen present. In connection with the use of seaweeds as cattle food, and fertilizer, nitrogen vies with potassium in importance.

The difference between the additive figures for cellulose (crude fibre), protein and salts (ash), and the total solid content as determined by the moisture, may be taken as polysaccharide. A complete proximal analysis of six species of algae is thus presented in the following experimental section.

Experimental

PREPARATION OF SAMPLES

The samples used in the following analyses were composed of a number of plants collected at the same time from the same place. These data have been noted for each sample. Individual collections have been kept separate

on account of seasonal and ecological variations; a number of plants have been included in order to make the samples as uniform and representative as possible. For identification of species reference has been made to FARLOW's "Marine algae of New England." (7).

To lessen the effect of autolysis and bacterial action, the time elapsing between collection and completion of drying has been cut down to a minimum. In order to approach as nearly as possible to the ideal condition, the plants were kept in sea water until ready for treatment. After removal from the water, as much of the surface moisture as possible was removed by shaking or blotting, and the plants at once ground in a food chopper. To insure that each collection should have the same percentage of surface moisture was an impossibility so that a fairly wide variation in moisture determinations of the same species was foreseen, and table I represents averages.

The advisability of making moisture determinations on minced material might be questioned on the ground that moisture would be pressed out in the grinding. This was found to be true in very few cases, however, and since what little press juice was lost contained considerable solid matter the error introduced was accepted as slight. The chief reason for grinding the plants while fresh and moist is the ease with which it can be accomplished then. Many of them are impossible to grind once they have dried.

The minced material was dried, either in the oven below 100° C. or in the sun. When thoroughly dry it was again put through the chopper until finely powdered. In this form the samples were preserved; and just before using, a portion was dried to constant weight and kept in a desiccator.

TABLE I

MOISTURE CONTENT OF MARINE ALGAE

SPECIES	MOISTURE
	per cent.
Scytosiphon lomentarius (a)	91
Laminaria longicruris (a)	89
Porphyra laciniata (c)	86
Rhodymenia palmata (a) and (b)	85
Delessaria sinuosa (a)	83
Chondrus crispus (b)	77
Fucus vesiculosus (a) and (b)	76
Ascophyllum nodosum (c)	70
Gigartina mamillosa (c)	68

(a) Collected at St. Andrew's, New Brunswick, in June.
(b) Collected at St. Andrew's, New Brunswick, in July.
(c) Collected at Halifax, Nova Scotia, in November.

Moisture determination

These determinations were made by drying a weighed sample of the minced plant, usually 10–20 grams, to constant weight in the oven below 100° C. The loss of weight was calculated as moisture. Thirty hours were usually sufficient for complete drying. Percentages of moisture in fresh samples examined are presented in table I.

Ash determinations

Moisture-free samples of 3 to 4 grams were used in all cases and ignited in an electric muffle furnace at 575°–600° C. for five hours. In table II will be noted the analyses of the ash of two species hitherto unrecorded. That of *Laminaria longicruris* is remarkably high. My average figure for *Laminaria* of 21.9 per cent. agrees very well with that of Reed of 21.1 per cent. For the most part the agreement is good and does not show any marked difference between the salt content of northern Canadian forms and of those of Hawaii (Reed) and of France (Vincent, **26**).

TABLE II

Comparative table of ash values as percentage of dry weight

Species	Results of present investigation	Previous results	By whom reported
Laminaria longicruris (a)	33.2	—	
Laminaria andersonii	—	26.5	Hoagland (11)
Laminaria (average of several species) (d)	21.9	21.1*	Reed (20)
Chondrus crispus (a)	27.7	14.2*	Reed (20)
		14.6	Haas (9)
		24.9	Vincent (26)
Rhodymenia palmata (a)	26.9	36.6	Vincent (26)
Gigartina mamillosa (c)	22.5	—	
Fucus vesiculosus (a)	20.7	22.2	Vincent (26)
Fucus serratus	—	26.3	Vincent (26)
Ascophyllum nodosum (b)	22.2	23.6	Vincent (26)
Porphyra laciniata (a)	15.7	10.37*	Reed (20)

* Miss Reed's figures are for air dry material, not dry weight.
(a) Collected at St. Andrew's, New Brunswick, in July.
(b) Collected at Halifax, Nova Scotia, in November.
(c) Collected at Digby, Nova Scotia, in September.
(d) Collected at Halifax, Nova Scotia, in September.

Potassium determination

Potassium determinations were made on the ash residues prepared as previously described, and results calculated to dry weight. The method

used was that of KRAMER and TISDALL (13) for the estimation of potassium in blood serum. A definite weight of each ash, 0.10 to 0.25 gram, was dissolved with the aid of 1 or 2 cc. of sulphuric acid, and the solution made up to 100 cc. These solutions were used to replace the serum of the original method. The results given in table III are the average of duplicate analyses, only one of which varied more than 0.03 cc. in the final titration.

When the figures in table III are compared the conclusion may be drawn that potassium is richer in the specimens which I have analyzed than in those from other water. *Rhodymenia palmata* is a striking instance of this. The only possible exception is that of *Fucus vesiculosus* on the basis of BARLOW'S analysis. I have been unable to find any analysis in the literature for the potash content of *Gigartina* or *Porphyra*. The relationship between content of ash and of potassium will be seen in table VII.

TABLE III

COMPARATIVE TABLE OF POTASSIUM VALUES AS PERCENTAGE OF DRY WEIGHT

SPECIES	RESULTS OF PRESENT INVESTIGATION	PREVIOUS RESULTS	BY WHOM REPORTED
	per cent.	*per cent.*	
Rhodymenia palmata (a)	12.22	2.42	WHEELER & HARTWELL (28)
		4.48	VINCENT (26)
Laminaria longicruris (a)	7.33	—	
" *digitata*		5.40	HENDRICK (19)
" "		5.49	WHEELER & HARTWELL (28)
" *andersonii*		2.48	BURD (3)
" *saccharina*		2.08	WHEELER & HARTWELL (28)
Chondrus crispus (a)	4.58	1.53	WHEELER & HARTWELL (28)
Fucus vesiculosus (a)	3.39	6.29	BARLOW (2)
		2.24	VINCENT (26)
		1.50	WHEELER & HARTWELL (28)
Gigartina mamillosa (b)	3.18	—	
Porphyra laciniata (a)	2.69	—	

(a) Collected at St. Andrew's, New Brunswick, in July.
(b) Collected at Digby, Nova Scotia, in September.

IODINE DETERMINATIONS

Iodine was determined in moisture-free samples by KENDALL'S method (12), the amount of sodium hydroxide used for fusion, however, being reduced by half.

There is in the literature a very extensive series of iodine determinations. Moreover in these analyses there is a high percentage of variation, partly due to seasonal variations and partly to technical errors. I report

analyses for several species hitherto undetermined. Table IV reveals many points of close agreement. The Canadian forms do not show any consistently higher level of iodine content. *Laminaria longicruris* is notably low in iodine for its genus.

TABLE IV

COMPARATIVE TABLE OF IODINE VALUES AS PERCENTAGE OF DRY WEIGHT

SPECIES	RESULTS OF PRESENT INVESTIGATION	PREVIOUS RESULTS	BY WHOM REPORTED
	per cent.	*per cent.*	
Rhodymenia palmata (a) & (c) ...	0.0231	0.122	CAMERON (4)
		0.7120	STANFORD (23)
		0.081	VINCENT (26)
Prophyra laciniata (a)	0.0085	——	
" *vulgaria*	——	0.009	CAMERON (4)
Chondrus crispus (b)	0.0736	0.053	CAMERON (4)
Gigartina mamillosa (c)	0.1237	0.016	CAMERON (4)
Ascophyllum nodosum (b)	0.1362	0.0572	STANFORD (23)
		0.084	VINCENT (26)
Fuscus vesiculosus (a)	0.0127	0.0297	STANFORD (23)
		0.015	WEIBULL (21)
		0.028	VINCENT (26)
Laminaria longicruris (a)	0.0737	——	
" *digitata* (d)	0.349	0.45	DOHERTY (6)
		0.5	HENDRICK (10)
		0.4535 (stipe)	STANFORD (23)
		0.2946 (frond)	STANFORD (23)
Agarum turneri (d)	0.097	——	
" *fimbriatum*	——	0.09	TURRENTINE (25)
		0.022	CAMERON (4)
Alaria esculenta (d)	0.101	——	
" *lanceolata*	——	0.06	TURRENTINE (25)
Halosacchion ramentaceum (d) ...	0.029	——	
" *glandiforme*	——	0.006	CAMERON (4)

(a) Collected at St. Andrew's, New Brunswick, in June.
(b) Collected at Halifax, Nova Scotia, in November.
(c) Collected at Digby, Nova Scotia, in September.
(d) Collected at Halifax, Nova Scotia, in September.

NITROGEN DETERMINATIONS

Percentage of nitrogen in moisture free samples was estimated by Kjeldahl's method, and does not therefore include nitrate nitrogen. The figures obtained for nitrogen are in agreement with those of previous investigators and require no comment in themselves. If it is assumed that this nitrogen comes from protein and is calculated as such the value obtained

represents only a small portion of the organic matter present. This sharply differentiates the metabolism involved.

TABLE V

COMPARATIVE TABLE OF NITROGEN VALUES AS PERCENTAGE OF DRY WEIGHT

SPECIES	RESULTS OF PRESENT INVESTIGATIONS	PREVIOUS RESULTS	BY WHOM REPORTED
	per cent.	*per cent.*	
Rhodymenia palmata (a)	3.71	2.71	WHEELER & HARTWELL (28)
		1.84	VINCENT (26)
Porphyra laciniata (a)	3.61	5.55*	REED (20)
Gigartina mamillosa (b)	3.54	—	
Laminaria longicruris (a)	1.91	—	
" *digitata*	—	1.74	WHEELER & HARTWELL (28)
		1.23	HENDRICK (10)
		1.63	WHEELER & HARTWELL (28)
" *saccharina*	—	2.4	HOAGLAND (11)
" *andersonii*	—	2.4	BURD (3)
Laminaria (average of 7 species)	—	0.89*	REED (20)
Chondrus crispus (a)	1.65	2.24	VINCENT (26)
		2.26	WHEELER & HARTWELL (28)
		1.5 *	REED (20)
Fucus vesiculosus (a)	1.37	2.43	VINCENT (26)
		1.61	WHEELER & HARTWELL (28)
		2.29	BARLOW (2)
		0.99	HENDRICK (10)

* REED'S figures are for air dry, not moisture free samples.

(a) Collected at St. Andrew's, New Brunswick, in July.

(b) Collected at Digby, Nova Scotia, in September.

CRUDE FIBER DETERMINATION

Crude fiber was determined according to the method given by LEACH (**16**) for its estimation in cereals. There have been few determinations of crude fiber in algal forms. The values are fairly constant for the species analyzed and they are consistently low. This absence of the cellulose complex among the red and brown forms can be taken as another characteristic of their metabolism.

Table VI presents a comparison of crude fiber values of a number of species, and in table VII will be found a summary of the constituents of six species found abundantly on the coast of the Maritime Provinces of Canada. By the addition of the values for ash, crude fiber and protein (N x 6.25) the difference may be taken to be carbohydrate other than cellulose. Thus

50–60 per cent. of the total solid content of these algae is in this form which is probably mostly polysaccharide judging from personal experience.

TABLE VI

COMPARATIVE TABLE OF CRUDE FIBRE VALUES AS PERCENTAGE OF DRY WEIGHT

SPECIES	RESULTS OF PRESENT INVESTIGATIONS	PREVIOUS RESULTS	BY WHOM REPORTED
	per cent.	*per cent.*	
Fucus vesiculosus (a)	5.21	—	
Laminaria longicruris (a)	3.61	—	
" (average of 6 species)	—	6.7*	REED (20)
" *andersonii*	—	10.4	HOAGLAND (11)
Porphyra laciniata (a)	2.75	—	
Chondrus crispus (a)	2.15	2.2*	REED (20)
Gigartina mamillosa (b)	2.05	—	
Rhodymenia palmata (a)	1.50	—	

* REED'S figures are for air dry, not moisture free samples.
(a) Collected at St. Andrew's, New Brunswick, in July.
(b) Collected in Digby, Nova Scotia, in September.

TABLE VII

COMPOSITE TABLE

SPECIES	MOISTURE*	ASH	POTASSIUM	IODINE	CRUDE FIBER	NITROGEN	COMMON NAME
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	
Laminaria longicruris	88.5	33.2	7.35	0.0737	3.61	1.91	Kelp
Fucus vesiculosus	76.4	20.7	3.39	0.0127	5.21	1.37	Rockweed
Rhodymenia palmata	85.4	26.9	12.22	0.0231	1.50	3.71	Dulce
Porphyra laciniata	85.7	15.7	2.69	0.0085	2.75	3.61	—
Chondrus crispus	76.8	27.7	4.58	0.0736	2.16	1.65	Irish moss
Gigartina mamillosa	78.0	22.5	3.18	0.1237	2.05	3.54	—

* All analyses except that of moisture are expressed as percentage of dry weight.

Discussion

The influence of ecological factors on the composition of marine algae must first be stressed; and this is a fact which, unfortunately, has not been appreciated adequately by previous investigators. Habitat and season of collection age of plant (**8**), temperature and salinity of surrounding water,

and depth of submergence, all have an influence on the nature and amount of constituents present in the plant. For example, SAUVAGEAU (**22**) compared the efflorescence on two specimens of *Rhodymenia palmata;* one was collected on the coast of France, the other on the coast of Iceland. On the plant from Iceland he found both potassium chloride and mannitol; on the plant collected at Roscoff, potassium chloride but no mannitol. LAPICQUE (**15**) found *Laminaria flexicaulis* to contain two per cent. soluble carbohydrates in the early spring as compared with eighty-one per cent. in the late summer. Mineral salts also vary with the seasons. Furthermore, the percentage of the same element, *e.g.* potassium or iodine, varies in different parts of the same plant. In making comparisons of analyses of the same species as well as of different species these facts must be constantly considered. For the reason that there is a large unavoidable error introduced in the determination of the moisture content of algae, it is highly desirable that analyses of other constituents be expressed in terms of the dry weight.

As might be expected the species having the highest ash values are also those with the highest content of potassium. From table VII it is apparent that potassium constitutes a considerable portion of the ash. This is especially true for *Laminaria longicruris* and *Rhodymenia palmata.*

The values for iodine are difficult to compare because of the readiness with which it is lost in preparatory handling. It will diffuse readily from the dead plant, (DANGEARD, **5**) and is volatile in the free form, (SAUVAGEAU, **22**). The apparent presence of iodine in the free form, as iodide, and in organic combination, in varying degrees (KYLIN, **14**) makes the problem a difficult one. The volatility of potassium iodide at high temperatures introduces a possible technical error.

The use of algae and their products as food, though inconsiderable on the North American continent, is extensive in other parts of the world. Their value as such is dependent on the mineral salts and proteins which may be present. SAIKI (**21**) has shown that the carbohydrates present are very difficult to hydrolyze by means of enzymes, and hence are almost indigestible to man; and only slightly digested even by ruminants. Regarding the proteins of algae little is known and much work needed. HOAGLAND (**11**) gives nitrogen values from 0.98 to 2.67 per cent. dry weight of the Pacific Coast kelps, of which 13 to 37 per cent. is in non-protein form. Therefore the maximum calculated on this basis would be 14.5 per cent. protein. In the present investigation a maximum nitrogen value of 3.71 per cent. dry weight, (exclusive of nitrates), was found in *Rhodymenia palmata,* which, converted by the factor 6.25, gives 23.19 per cent. protein. KYLIN (**14**) reports that ammonium nitrate occurs in all, nitrates in some, but not in others.

STEWART (**24**) testing the efficacy of algae as a fertilizer, found the nitrogen present to be readily available in some species, less readily in

others. The fact that 50 per cent. of the dry weight of organic matter is capable of furnishing humus to the soil (**3**) is an added advantage in a fertilizer. Potassium, which is essential to a good fertilizer, is present chiefly as chloride and sulphate (**1**).

It has been difficult to find similar analyses reported for the same species with which the present work was concerned. This is due to the fact that they are not indigenous to the Pacific Coast where any work done on this continent has been carried on. Where it was found impossible to compare with the same species, other species of the same genus have been included, if available. On the comparative tables relative to each determination, quotations have been given from a number of workers, in order to emphasize the fact that there is such a wide variation in the results, which might be considerably lessened if all the limiting factors were known.

Summary

Several species of algae common to the North Atlantic Coast have been analyzed for their content of moisture, ash, potassium, iodine, protein and crude fiber.

Moisture determinations on nine species were found to average 80 per cent., with a maximum of 91 per cent., and a minimum of 68 per cent.

Ash determinations averaged 25 per cent. of the dry weight; maximum in *L. longicruris* of 33.2 per cent., minimum in *P. laciniata* of 15.7 per cent.

Potassium determinations on six species gave an average content of 5.57 per cent. of the dry weight; maximum in *R. palmata* of 12.22 per cent., minimum in *P. laciniata* of 2.69 per cent.

Iodine has been determined in twelve species the average content of which is less than 0.1 per cent. of the dry weight. The maximum of 0.349 per cent. was found in *L. digitata,* a minimum of 0.0085 per cent. in *P. laciniata.*

Nitrogen determinations were made on six species, three of which were much higher than the others, and calculated as protein (N x 6.25) would average 22.6 per cent.

Crude fiber determinations were made on six species and only one found to be above 5 per cent.; the majority are less than half of this amount.

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RELATION OF PHOTOTROPISM TO THE WAVE-LENGTH OF LIGHT*

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(WITH TWO FIGURES)

Many conflicting theories and statements appear in plant physiological literature regarding phototropism. Such conditions frequently exist in a young science where much of the earlier work is qualitative. In many of these early experiments dealing with the bending of plant stems toward light, the lights used were such that it was impossible to determine accurately whether the color or the intensity was the predominating cause of the bending. The relative influence of the different colors, or wave-lengths, could not be determined because different intensities were used. Both of these light factors must be considered, as a greater value of one may off-set the lesser value of the other. In order to determine which colors are most effective in phototropic bending, it is necessary to use wave-lengths of equal intensities.

One method of evaluating the wave-length effects is described by HURD.[1] Wratten light filters were used between the light source and the fucus spores and rhizoids with which this work was performed. The intensities were made equal by increasing or decreasing the distance of the electric arc from the measuring instrument. PARR,[2] working with *Pilobolus* under carefully controlled conditions, found that light in all regions of the visible spectrum brought about responses in this plant. The presentation time decreased gradually from red to violet, with no indication of intermediate maxima or minima. This work appears to be at variance with statements appearing even in recent text books on plant physiology, that the more highly refrangible rays of light are most effective in phototropic movement with the effect diminishing from blue to yellow, and again increasing in the red and infra-red.

PRIESTLEY[3] has recently attempted to give a rational explanation to the phenomenon of phototropism. He shows that phototropic curvature in coleoptiles is consistent with the "light-growth" hypothesis, in spite of

* The investigations herein described have been carried out in cooperation with the University of Maryland.

[1] HURD, ANNIE MAY. Some orienting effects of monochromatic lights of equal intensities on fucus spores and rhizoids. Proc. Nat. Acad. Sci. 5: 203–204. 1919.

[2] PARR, ROSALIE. The response of *Pilobolus* to light. Ann. Bot. 32: 177–205. 1918.

[3] PRIESTLEY, J. H. Light and growth. New Phyt. 24: 271–283. 1925, and 25: 145–170; 213–247. 1926.

many seemingly discrepant experiments. The amount of light required to induce phototropic curvature in normal light-grown shoots is greater, and must be continued longer, than that required to bring about similar curvatures in *etiolated* shoots. It is thus evident that light affects normal and etiolated shoots quite differently. Briefly, the mechanism of bending in etiolated shoots as discussed by PRIESTLEY, is as follows: The walls of the cells making up the tissue contain fat and protein. These substances prevent the ready passage of sap and water from the vascular system to the meristematic tissue which, under favorable conditions, is capable of rapid growth. BLAAUW points out the similarity between light and its photochemical effect on a photographic plate on the one hand and an etiolated coleoptile on the other. Relatively small quantities of light produce photochemical actions on these shoots. Protein and fatty materials disappear from the cell walls, with the latter substances migrating mainly to the cuticle. The passage way between the meristematic cells and their water and food supply is opened up. In the words of PRIESTLEY, "Increased surface growth now ensues. Growth as a whole may be as active as ever on the more brightly lit side of the etiolated shoot, but it is differently distributed. More cells are added to the surface of the stem and leaf, and less proportionately contributed to the inner layers of the shoot axis. The result is, therefore, in the aggregate, a retardation of growth in length on the illuminated side, and a positive phototropic curvature."

Two classes of explanations for the modification in growth rate have been proposed: First, those assuming the effect to be predominantly due to a local temperature change brought about by unsymmetrical absorption of radiant energy; second, those postulating some type of photochemical action. In this second group would fall both those theories involving a local and direct photochemical change at the point of absorption of light, and those assuming a type of hormone action. The first group, involving a purely thermal hypothesis, would lead to a prediction of phototropic bending for all regions of the spectrum where the radiant energy could be absorbed by the cell materials. Infra-red for all wave-lengths longer than 1.1 μ is absorbed by the water, even in relatively small layers. The remainder of the cell materials would absorb light pretty generally through the entire visible spectrum. Where etiolated plants are used, with the absence of chlorophyll, a very selective action would not be expected. In the case of a photochemical hypothesis, however, one might have a highly selective type of absorption, either showing maxima and minima, or beginning weakly at some particular wave-length and increasing towards the shorter wave-lengths or blue end of the spectrum. Such selective absorption in the visible or ultra-violet is characteristic of electronic changes in energy within the molecule.

In the present experiment it was proposed to determine whether or not selective regional phototropic responses (*i.e.*, to different colors) would be found for equal intensities. Second, to determine, in case of selective responses whether the effect of different colors could be off-set by modification in intensities. Such an experiment would crucially decide between the two classes of hypotheses.

The general underlying idea of determining the relative influence of colors in a differential manner by exposing the plant to two different colors from opposite sides was proposed earlier by JOHNSTON.[4] The present experiment was simply an elaboration of this proposed method, wherein particular attention has been given to the physical quantities involved. The wave-length regions have been restricted by filters whose transmission curves were definitely determined. The intensities were measured by means of an especially constructed thermocouple and galvanometer system of high sensitivity, with which accurate determinations could be made over an unusually large range of relative intensities.

The details of the apparatus are as follows: The thermocouple-galvanometer system constitutes essentially a blackened thermometer whose rise in temperature above the surrounding room temperature is proportional to the amount of radiation falling upon it, and is practically independent of the wave-length of the radiation. The rise in temperature was indicated by a galvanometer deflection which was read by the displacement of a small band of light along a metric scale. The system consisted of a single closed electrical circuit of which the d'Arsonval galvanometer coil was a part. Where dissimilar metals are used in a circuit, the unequal heating of the points of contact causes an electromotive force. For small differences in temperature this electromotive force, and the current arising, is proportional to the difference in temperature.

The galvanometer coil and most of the circuit were constructed of copper. The remainder of the circuit was made up of a short length of fine bismuth wire and a short length of bismuth-tin alloy. The juncture of the copper with the bismuth, and the juncture of the copper with the bismuth-tin were each maintained at room temperature. To the point where the bismuth joins the bismuth-tin wire, a blackened receiver was attached. Radiant energy falling upon such a receiver raises its temperature above that of the junctures to the copper, and so causes the current. The alloy of bismuth-tin was made up of 95 per cent. bismuth and 5 per cent. tin. It is interesting to note that this small percentage of tin yields a material of opposite thermoelectric characteristics to pure bismuth.

In the choice of materials for construction of such a thermocouple, not

[4] JOHNSTON, EARL S. A plant photometer. Plant Physiol. 1: 89–90. 1926.

only the thermoelectric power, but also the resistance and the thermal conductivity should be considered. This combination of metals is the best known at the present time. The wire used was about 25 microns in diameter and between 2 and 3 mm. long. The receiver was a circular piece of platinum foil about 2 mm. in diameter. The thermocouple was then placed in a vacuum of better than 10^{-4} mm. pressure thereby serving a double purpose. First, the sensitivity was increased by the removal of convection loss, and second, small disturbances which ordinarily would reach the thermocouple through convection were eliminated. When radiant energy is focused upon such a receiver by means of a lens, it is equivalent to greatly increasing its area, and thus yielding a greater response than an actual increase in area would give, because the thermal losses would be increased at the same time. It is necessary, however, that the same effective area be used throughout the experiment. Because of the very great range of intensity to be observed, it was necessary to use a number of resistances in series and parallel, in order to change the sensitivity by several known factors.

The plant photometer box was 238 cm. in length and 30.5 cm. wide. It was divided into five compartments instead of three as in the earlier experiment. The end, or lamp compartments, were 59 cm. long and 59 cm. high, while the other three were 44 cm. high. The central, or plant compartment (87 cm. in length), was insulated from each lamp compartment by a filter chamber 10 cm. wide. Before the light could reach the plant from either side, it was passed through a water filter immediately surrounding the lamp, through a plate glass window, then through the filter chamber which was cooled by a stream of air, and finally through the desired color filter and a water cell, one window of which was made of heat absorbing glass. This water cell was set at an angle to avoid reflecting light to the plant from the opposite side of the plant chamber.

By the introduction of the cooled filter compartments between the lamp compartments and the plant chamber, heat necessarily arising in the lamp chambers was prevented from reaching the plant compartment. By means of the water cell with the heat absorbing window on the lamp side, only visible radiation was allowed to reach the plant. Water absorbs all the radiation in the infra-red longer than 1.1 μ, while the heat absorbing filter cuts out the near infra-red region between 1.1 μ and the visible. In the plant chamber the seedling was surrounded by a double-walled glass cylinder, the space between the walls being filled with water and the entire cylinder slowly rotated around the plant axis in order to equalize temperature conditions in the immediate environment of the seedling. The interior walls of all five chambers were painted a dull black. A general view of the photometer box is shown in figure 1.

The coleoptile of the oat was selected for these experiments. The seeds were germinated between glass plates covered with wet filter paper. The plates were so arranged in moisture chambers that the seedlings grew vertically. The seedlings were carefully selected for straightness and transferred to small Erlenmeyer flasks fitted with cork stoppers. Each seedling was supported by means of a little cotton in a small hole of the stopper. The flask was filled with distilled water so that the roots were entirely immersed. By means of a cross hair in a telescope the seedling was adjusted to a vertical position in the plant compartment between the light filters.

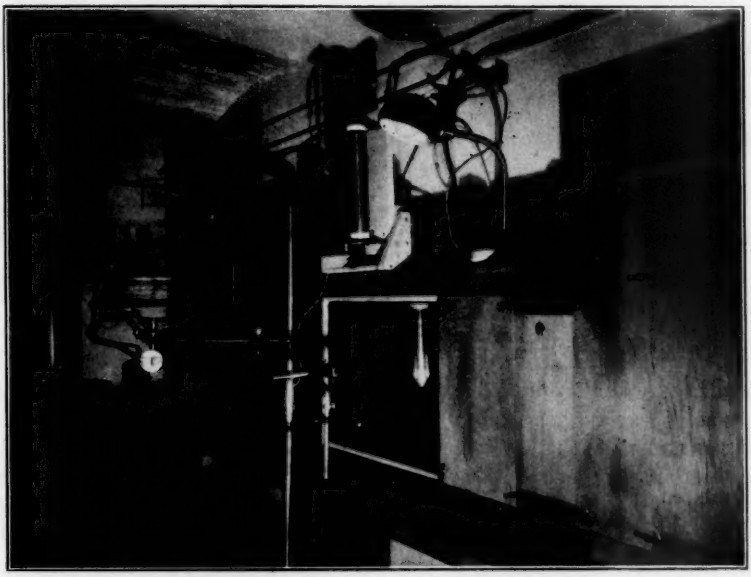

FIG. 1. General view of plant photometer with door of plant chamber removed to show double-walled glass cylinder in which seedling was grown.

In conjunction with the heat absorbing cell, four different light filters (2″ x 2″) were used in these preliminary experiments. Three were Wratten filters numbers 24 (red), 61 (green), 47 (blue) made by the Eastman Kodak Company, and one a "heat resisting yellow" (yellow shade) made by the Corning Glass Company.

The curves presented in figure 2 indicate the transmission of these filters. From these curves it will be observed that the red filter transmits freely all wave-lengths greater than 6000 Å. and absorbs all wave-lengths shorter than 5800 Å., in fact, cutting off practically all

wave-lengths shorter than 5900. The yellow filter transmits freely all wave-lengths longer than 5400 and cuts off all wave-lengths shorter than 5200, effectively removing all wave-lengths shorter than 5350 Å. The green filter shows a maximum transmission at about 5100, practically cutting off all wave-lengths beyond 6000 on the red side, and 4800 on the blue side, with no measurable transmission shorter than 4700. The blue filter transmits effectively a region from 3900 to 5000.

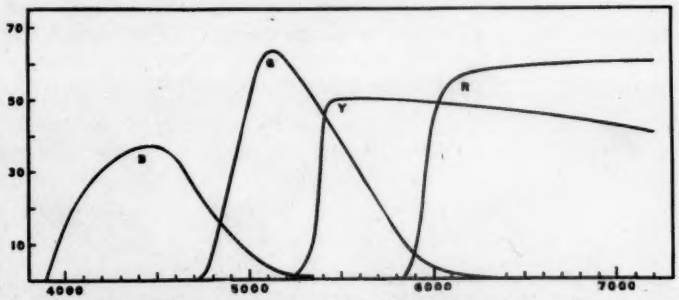

FIG. 2. Curves representing percentages (ordinate) of transmission of the light filters; blue (B), green (G), yellow (Y), and red (R). Wave-lengths are expressed in Ångström units along the abscissa.

The results obtained were as follows. In the first place where the plant was exposed to radiation from only one side with the water cells all removed, and a filter introduced which absorbed all the visible light, no phototropic bending could be observed. In other words, infra-red radiation between the visible and 2.5 μ produced no phototropic bending which could be observed. Second, with the heat absorbing filters together with the red filter and no light from the opposite side, no measurable phototropic bending could be detected. Hence no wave-lengths longer than 6000 Å. could be found to produce a measurable phototropic effect. Similarly, with the yellow filter substituted for the red filter, the plants were grown with no opposing light. In this case, a noticeable bending was soon apparent. Hence a phototropic influence is certainly to be found in the region between 5200 and 6000 Å units, and probably in the narrow region between 5350 and 5900 Å. This is the region usually termed yellow. It is impossible from the present experiment to say which portion of this region may be effective. For the green and blue filters the unbalanced phototropic effect was very marked, the bending taking place in a very few minutes. The first differential balance was observed with the yellow light on one side and the green light on the other. A balance was actually secured, however, only when the yellow light was 1,000 times more intense than the green light as determined by the non-selective thermocouple-galvanometer mea-

surements. In the same way a balance was reached between the green and blue when the green light was 30 times more intense than the blue.

In each experiment a single seedling was used. The general procedure was to place the seedling between two different light sources and, after a time interval, observe the coleoptile through the telescope in order to determine any growth curvatures. If, for example, the seedling was exposed between the blue and green filters and a distinct bending toward the blue was shown at the end of half an hour, the lights were so adjusted by position and current controlled by rheostats, that the green intensity was increased and the blue decreased. Another seedling was then placed in the photometer and the experiment repeated. After several trials a balance point was reached where the effect of one light was neutralized by the effect of the other, and the plant continued to grow in a vertical position even after an exposure of several hours. When this balance point was reached the seedling was removed and the especially constructed thermocouple placed in the position of the plant. The relative intensities of the lights were then measured by means of the galvanometer.

It is of interest to note that repetition of the experiment yielded balance points differing by less than 5 per cent. from the previous experiment. Such reproducibility is somewhat unusual in biological measurements. It suggests that by this method one is observing a characteristic of some underlying photochemical reaction. The absence of effect in the red and infra-red together with a very sharp increase in passing from yellow to green, and the subsequent rise in the blue, is typical of an electronic photochemical reaction. The experiment must be regarded, therefore, as crucial evidence against a purely thermal theory and strong support for a photochemical theory.

The results seem of sufficient importance to justify a more elaborate experiment wherein narrower spectral regions are used, that is, more restricted color ranges, thus enabling one to determine the phototropic effect at many points through the spectrum. For this purpose a monochrometer must necessarily be used. In such an experiment it seems desirable also that the phototropic influence of all the wave-length regions be determined in terms of a single comparison band. The intensity of the comparison light could be varied to counterbalance successively the phototropic influence of each band or color, taken preferably all at the same intensity. The experiment should also be carried into the ultra-violet in order to gain the additional information which may throw some light upon the nature of the photochemical reactions involved.

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VARIATIONS IN THE O_g OF PLANT TISSUES

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(WITH FOUR FIGURES)

The physical chemists have expressed widely different views regarding the nature of osmotic pressure, but while advancing different theories in explanation of the phenomenon, they agree in the quantitative expression of the so-called pressure. If the osmometer containing the solution in question be placed into the solvent, the difference of pressure on the solution and the solvent expresses numerically the osmotic pressure of the solution in the osmometer, when a condition of equilibrium exists, *i.e.*, when no solvent flows in either direction, from or to the solution through the membrane.

The definition of osmotic pressure is illustrated in figure 1. If the

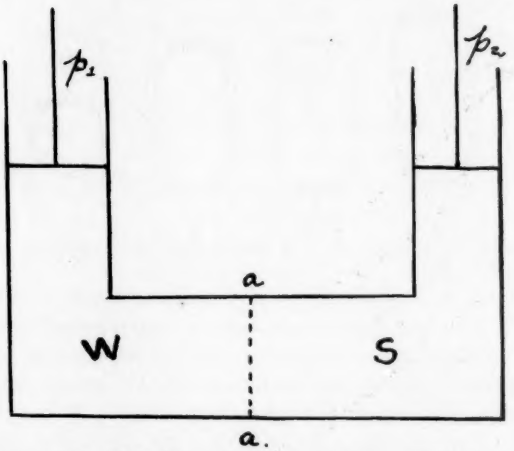

FIG. 1. A diagrammatic illustration of osmotic pressure. *W* is the solvent, *S* is the solution, *a* is the membrane; p_2 is the pressure on the solution, p_1 is the pressure on the solvent. In a condition of equilibrium p_2-p_1 is the numerical expression of the osmotic pressure.

pressure on the solution (p_2) is too small solvent (W) will flow through the membrane (a) into the solution (S). If it is too great, solvent will flow from the solution (S) into the solvent (W), passing through the membrane in the reverse direction. If the solvent fails to flow in either direction a condition of equilibrium exists at the membrane; under this condition, the difference of the mechanical pressures p_2 and p_1 expresses the osmotic pressure of the solution (S).

$$P = (p_2 - p_1)$$

There is nothing ideal about this quantity, it is very real; it is expressed in atmospheres.

This quantity is not under discussion in this paper and is mentioned here only to emphasize the fact, because some confusion exists about the terms in current literature on this subject.

It is practically impossible to measure the osmotic pressure of the normal cell sap directly. Some investigators measure the osmotic pressure indirectly of sap expressed from plant tissues, by the indirect cryoscopic method. This method can hardly yield results that will be helpful in determining physiological activities in plant tissues. I think this will appear from the results which are presented in this paper.

When a cell is placed into a solution of sufficient concentration, plasmolysis occurs (fig. 2). The receding film of plasm can readily be de-

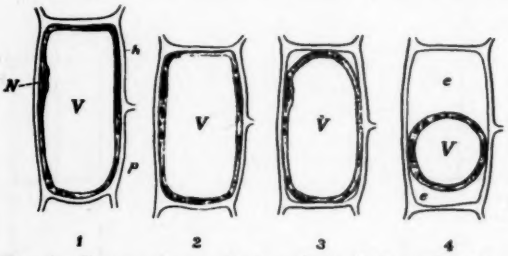

FIG. 2. Various stages of plasmolysis (After DEVRIES).

tected even at incipient plasmolysis, if the illuminating conditions are favorable. If the incipient stage of plasmolysis remains constant, the relative concentration of the plamolysing agent is taken as a measure of the relative concentration of the sap within the cell at that stage. The concentration of the agent is expressed in gram molecular units (volumetric system). This quantity which expresses the relative concentration of the cell sap at incipient plasmolysis, is termed the *osmotic value* of the cell, at incipient plasmolysis; it is symbolized by O_g. This quantity should always be expressed in mols and not in atmospheres. If the equivalent atmospheres are to be expressed for any reason, evidently only such an agent should be employed for which the equivalent atmospheres of osmotic pressure have been accurately determined. For various reasons given elsewhere, cane sugar was employed as the plasmolysing agent in these studies, and when it became necessary for clearness, the formula for cane sugar was appended to the symbol for the osmotic value.

No attempt is made in this paper to draw conclusions about the relative concentration of the cell sap in the normal condition of the cell. The

object is to study variations in the value of O_g. When there is a variation, it indicates a variation in the *quantity* or the *nature* of the solutes in the cell sap, or a variation in both the nature and the quantity of the solutes.

Some investigators tried to show that there is an O_g gradient in the direction of the flow of water in the plant. URSPRUNG and BLUM showed that there is a gradient in the quantity which expresses the potential ability of the cell to absorb water (they call it suction force), and that the O_g varies irregularly. Without data on the osmotic values of the cell sap and the relative volumes of the cell in the normal condition at incipient plasmolysis and when saturated, no conclusions can be drawn about the potential ability of the cell to draw water. From this it is clear that the problems taken up here are quite different from the problems which URSPRUNG and BLUM discuss under the name of Suction Force studies.

The cells of a given tissue do not as a rule have exactly the same O_g. When fifty per cent. of the cells of a tissue are slightly plasmolysed, the concentration of the agent is taken as the average value; it is expressed as the O_g of the tissue. Numerous tests have shown that this value is reliable, *i.e.*, different tests made at short intervals under the same conditions on a given tissue, yield the same results.

Different tissues in a given plant have widely different O_g's. This will be clearly shown in the data that follow. Within limits the O_g of a given tissue is characteristic for that tissue. Assimilating tissues for example have higher values as a rule, than epidermal tissues. The range of variation of O_g in response to given factors, is not as great in the epidermal tissues as it is in the assimilating tissues. The O_g of the guard cells varies rapidly within wide limits, under the influence of certain external factors. An example by way of illustration: During the months of March and April, a considerable number of O_g measurements were made for the tissues of ivy leaves all of which were taken from the same plant. The highest value recorded for the epidermis was 0.775; the lowest 0.6; the values found most frequently was 0.65. The highest value recorded for the palisade parenchyma was 1.25, the lowest 1.05, and 1.1 the most frequent value. For the guard cells the lowest was 0.6, the highest 0.825 and the most frequent 0.65.

In table I the O_g is recorded for the lower epidermis, the guard cells and the spongy parenchyma of twenty different plants. The O_g seems to depend upon the nature of the plant as well as upon the environment in which the plant grows. The table has been arranged in such a way as to indicate the relation of the O_g to the nature of the plant. Not more than ten minutes intervened between the measurements of the O_g for the different tissues of a given plant. For any given plant the epidermal tissue invariably showed a lower value than the spongy parenchyma; the guard cells had nearly the

TABLE I

THE O_g ($C_{12}H_{22}O_{11}$) OF THE TISSUES OF LEAVES

HERBACEOUS				WOODY			
PLANT	LOWER EPIDERMIS	GUARD CELLS	SPONGY PARENCHYMA	PLANT	LOWER EPIDERMIS	GUARD CELLS	SPONGY PARENCHYMA
Solanum nigrum	0.425	0.675	0.575	*Ligustrum vulgare*	0.65	0.75	0.975
Crambe maritima	0.375	0.65	0.65	*Hybiscus syriacus*	0.6	0.55	0.8
Hesperis matronalis	0.55	0.6	0.725	*Fraxinus excelsior*	0.775	0.825	1.1
Euphorbia lathyrus	0.25	0.275	0.575	*Cytisus laburnum*	0.675	0.9	1.075
Osmunda regalis	0.475	0.425	0.875	*Robinia pseudacacia*	0.525	0.7	0.9
Polygonum orientale	0.375	0.55	0.675	*Liriodendron tulipifera*	0.6	0.675	0.825
Musa sinensis	0.4	0.35	0.45	*Hedera helix*	0.55	0.575	0.675
Datura stramonium	0.3	0.425	0.55	*Crataegus oxyacantha*	0.55	0.575	0.85
Acanthus spinosus	0.475	0.525	0.625	*Castanea sativa*	0.575	0.625	0.775
Dioscorea batatas	0.35	0.375	0.6	*Populus nigra*	0.5	0.575	0.625

same value as the epidermal tissue in some cases, and widely different values in other cases. For example in *Dioscorea batatas* the value was 0.35 for the epidermis, 0.375 for the guard cells and 0.6 for the spongy parenchyma; in *Solanum nigrum* the value was 0.425 for the epidermis, 0.675 for the guard cells and 0.575 for the spongy parenchyma; among the woody plants *Hedera helix* and *Cytisus laburnum* illustrate the same point. The measurements were made on different days but always in the morning between 6:30 and 8:30. During the early hours of the day the influence of the heat and light was not yet as great as it would have been later in the day. The difference of the mean values for the herbaceous plants was 0.0875 between the guard cells and the epidermis, in favor of the guard cells; it was 0.2325 between the spongy parenchyma and the epidermis, in favor of the spongy parenchyma. The difference of the mean values for the woody plants was 0.075 between the guard cells and the epidermis, in favor of the guard cells, it was 0.261 between the spongy parenchyma and the epidermis, in favor of the spongy parenchyma. It is interesting to note that while the actual values are considerably higher in the woody plants than they are in the herbaceous, the differences of the mean values are about the same in the woody and the herbaceous plants. The average value for the epidermis was 0.3975 in the herbaceous plants and 0.6 for the woody; for the guard cells it was 0.485 in the herbaceous and 0.675 in the woody; for the spongy parenchyma it was 0.63 in the herbaceous, and 0.861 in the woody plants.

The results of the experiments, which were carried out to demonstrate the variation of the guard cells in response to the natural factors which influence the plants during the day, are recorded in table II. The values recorded for the guard cells were usually not much different from the value recorded for the epidermis of the same plant, because the readings were taken at an early hour, when the factors had not effected a great variation in the relatively short time of exposure. The measurements recorded

TABLE II

VARIATIONS OF O_g IN THE GUARD CELLS IN RESPONSE TO LIGHT

PLANT	No.	LOWER EPIDERMIS		GUARD CELLS		SPONGY PARENCHYMA		SHADED
		LIGHT	SHADE	LIGHT	SHADE	LIGHT	SHADE	
Sedum spurium	1	0.275	0.275	0.500	0.4	0.450	0.550	Naturally
Crambe maritima	2	0.375	0.375	0.950	0.625	0.550	0.550	By umbrella
Crambe maritima	3	0.375	0.375	0.950	0.500	0.550	0.550	By foil
Nymphaea alba	4	0.300	0.300	0.550	0.550	0.500	0.500	Naturally
Vinca major	5	0.75	0.75	1.250	0.950	0.750	0.750	By foil
Vinca minor	6	0.65	0.65	1.300	0.950	0.750	0.750	By foil

in table II were made in the evening. Some plants were exposed to the light during the day, while neighboring plants were shaded. Except in one case, the shaded plants showed considerably lower O_g in the guard cells than did the plants normally exposed to the sun. It is not surprising that the one exception (*Nymphaea alba*) behaved as it did, if the guard cells are understood to be a regulating tissue: The water supply is maximum at all times so that no high degree of regulation is necessary for the rate of transpiration. Plant no. 3, *Crambe maritima* showed the greatest variation, the plant in the shade showing a value of 0.45 mol less than the plant in the light.

A great many measurements that were made on the tissues of leaves, taken from various plants at different times of the same day, tend to show that the O_g of the epidermis does not vary much, if at all, during the day, no matter if the weather be fair or rainy; the guard cells vary considerably, particularly when the weather is fair; the spongy parenchyma varies more than the epidermis, though usually not as much as the guard cells.

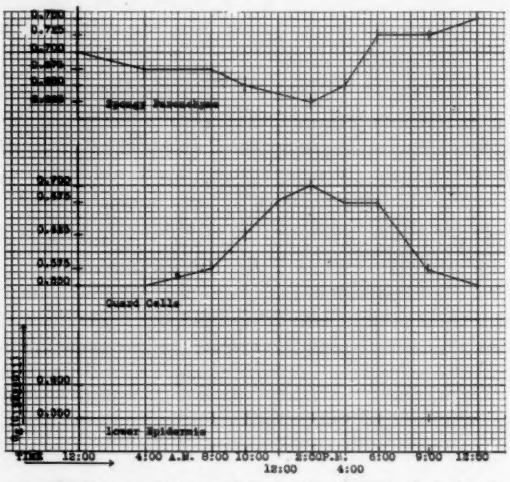

FIG. 3. Diurnal variations of O_g in the tissues of an ivy leaf.

In figure 3 the graphs of the results obtained on the leaf of *Hedera helix* are given by way of illustration of the variations which occur in the epidermis, the guard cells and the spongy parenchyma, during a period of 24 hours.

The value of the epidermis remained constantly at 0.550. The guard cells had an initial value of 0.550 at midnight and at 4:00 A. M.; then an increment set in, which continued at different rates up to 2:00 P. M., at

which time the maximum value was 0.700; the greatest rate of increment was between 8:00 A. M. and noon; the rate was less from 4:00 A. M. to 8:00 A. M. than it was from noon to 2:00 P. M.; the drop from the maximum value after 2:00 P. M. was decided and sharp until 4:00, when no variation occurred until 6:00 P. M., then the decrement was sharper than after 2:00 P. M.; a slower rate of decrement set in at 9:00 and continued until midnight when the initial value of 0.550 was reached.

The variations of the spongy parenchyma were quite different from those of the guard cells. The sense of the variation was usually opposite, and the rates were different. The initial value at midnight was 0.70; there was a decrement up to 4:00 A. M. and then the value remained constant up to 8:00 A. M. (at 0.675). From that time up to 2:00 P. M. there was a decrement with a greater rate of change from 8:00 A. M. to 10:00 A. M. than from 10:00 A. M. to 2:00 P. M. The lowest value at 2:00 o'clock was 0.625. From that time a considerable increment occurred until 6:00 P. M., when the value 0.725 was reached. The value remained the same until 9:00 P. M., when a further increment was experienced up to midnight; then the maximum was reached at 0.750, which was 0.05 mol higher than the initial value.

The facts that it had rained shortly before this experiment was begun, and that during the time of the experiment the barometer was high with sunshine during the day, might be helpful in interpreting these interesting results.

Other plants were examined and found to respond in the same sense, but not always in the same degree. Among these I wish to mention, *Vinca minor, Vinca major, Cydonia japonica, Evonymus japonica, Acer negundo, Musa sinensis, Canna, Datura stramonium, Nasturtium officinalis, Solidago canadensis, Taraxacum officinale, Plantago major, Paeonia officinalis, Caltha palustris, Sedum telephium, Sedum spurium, Sempervivum tectorum, Crambe maritima, Euphorbia lathyrus, Sinapis alba,* and *Cobaea scandens.*

As might be expected, the degree of variation was not the same in all of these plants. In *Taraxacum* for instance, the epidermis showed a slight decrement from 7:00 A. M. to 3:00 P. M. (from 0.55 to 0.525). The guard cells manifested a greater increment in *Taraxacum* than in the case of the ivy leaf just cited. At 7:00 A. M. the value was 0.55 and at 3:00 P. M. it was 0.775. The spongy parenchyma decreased its O_g from 0.8 at 7:00 A. M. to 0.7 at 3:00 P. M. In *Plantago major* the maximum for the guard cells was 1.05 at 2:30 P. M. and the minimum 0.75 at 6:00 A. M. Simultaneously the spongy parenchyma showed a maximum of 0.9 when the guard cells were at minimum, and a minimum of 0.8 when the guard cells were at maximum. *Saponaria ocimoides* failed to show a variation in the spongy parenchyma, as well as in the epidermis, even though the

weather was sunny. The guard cells varied as usual but the increment was 0.1, from 0.65 to 0.75.

Time does not permit further discussion of this interesting subject. I feel convinced that the facts adduced indicate that interesting variations of O_g occur in the plant under the influence of the factors of the natural environment, and that they deserve investigation along the lines suggested.

Annual variations as well as daily variations occur. The results obtained tend to show that some factors which failed to affect a tissue in a short time will eventually affect it after a longer time of exposure. This is particularly true for the epidermis. In figure 4 the graphs of results obtained on an ivy leaf are given.

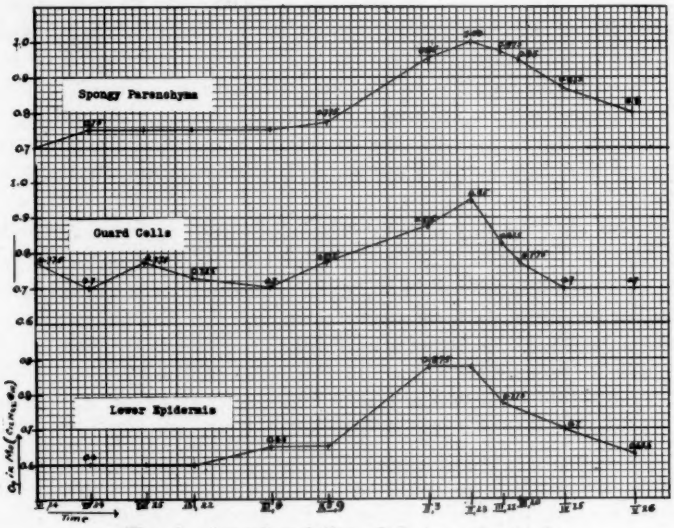

FIG. 4. Annual variation of O_g in an ivy leaf.

On June 24th the O_g of the epidermis was 0.6 mol, and on February 3rd of the following year it was 0.875 mol. The measurements recorded in the graph were made on the same plant which stood in garden soil, exposed to the weather for many years. All of the measurements were made at about 2:00 P. M. The guard cells were the least regular. This might be expected since they respond quickly to certain factors that might have influenced them for a short time, previous to the measurement. The variations in the spongy parenchyma are about as regular as those of the epidermis. It is particularly interesting that all of the tissues showed maximum values on February 23rd. The epidermis reached the maximum

of 0.875 mol on February 3rd already. The other two tissues continued to increase in O_g. The guard cells reached maximum value at 0.95, and the spongy parenchyma at 1.00 mol. The difference between maxima and minima were 0.275 for the epidermis 0.25 for the guard cells, and 0.3 for the spongy parenchyma. It is interesting that these differences are almost the same.

An explanation of these phenomena cannot be offered at present, and the facts are merely recorded for the consideration of other investigators. At some later time when the study of the different influences of certain factors on the O_g of plant tissues has been completed, it is hoped that an adequate explanation may be offered. It would be very helpful in this work to know more of the effect which these factors have on the nature and quantity of the solutes of the sap.

Summary

The O_g ($C_{12}H_{22}O_{11}$) is the osmotic value at incipient plasmolysis, when cane sugar is employed as the plasmolysing agent. It is expressed in molal concentration units. This quantity must not be confounded with the osmotic pressure, nor with the concentration of the cell sap.

Variations in the O_g for a given tissue can serve as an indicator of physiological activities in that tissue. Variations were studied in the epidermis, the guard cells, and the spongy parenchyma.

The O_g is characteristic for a tissue. It is greater in woody plants than in herbaceous plants.

The guard cells respond quickly and considerably to the influence of certain factors in the natural environment. The response in the spongy parenchyma was evident but usually not as rapid as in the guard cells. The response in the epidermis was usually negligible or slow.

The diurnal variation was negligible in the epidermis, considerable in the other two tissues. The character of the variation was different in the spongy parenchyma and the guard cells; at times the increments were opposed in direction.

The annual variations were evident in all three tissues, the O_g increased in all of them from the latter part of Spring to the end of February, when a rapid decrement set in, which continued up to Spring. The increment was least regular in the guard cells. The rate of increment was greatest in all of the tissues during the months of December and January.

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FACTORS MODIFYING THE TOXICITY OF PHENOL

WALTER S. EISENMENGER

(WITH ONE FIGURE)

Introduction

That various solids when added to toxic solutions have a detoxifying effect is not a new concept. TRUE and OGLEVEE (**4**) found that glass, filter-paper, etc., segregated the more toxic components of certain aqueous solutions, thus lowering the concentration of these toxins in other regions of the solvent. They found also that dilute strong poisons are more affected than strong weak ones. This seems to be true for both electrolytes and non-electrolytes.

It would seem reasonable to anticipate a decided detoxifying effect or increased concentration at the interface of the adsorbent if the solute lowered the interfacial tension of the solution appreciably and the adsorbent carried a charge opposite to that of the toxin in aqueous solution.

Silica gel has been used commercially for adsorption of certain gases, but perhaps is not so generally used as activated charcoal for solutions. In this study of the action of substances modifying the toxicity of phenol, silica gel was chosen, not for its particular efficiency, but for the reason that it resembles to a degree one or more components of the more heterogeneous materials of which the soil is composed and to which plants have adapted themselves as a medium.

It is not known to what extent phenol occurs in soils, but it is reasonable to suppose that it is present to an extremely slight extent, if at all. We do know that conjugated forms of it in minute quantities are at times thrown off as excretory products of animals and as such may find their way to the soil.

Plants have adapted themselves to substances found most frequently in soil waters, and in a general way we may state that those elements which have a high solution tension and are also most abundant in the earth's crust are the least toxic. For example, we may contrast the slight toxic effect of the relatively abundant elements, sodium, potassium, and calcium with the more toxic effects of the rather rare elements, caesium, lithium and barium.

The same seems true of non-electrolytes (although more exceptions can be noted). Phenol does not occur in nature to any perceptible degree, but it is quite soluble. It exerts a marked degree of toxicity. Acetic and formic acid molecules are less toxic than cinnamic and hippuric molecules,

(TRUE, 5). Methyl and ethyl alcohol are less toxic than normal amyl, or normal hexyl alcohol, (EISENMENGER, 2).

The objects of the present study were: (1) to determine the relative toxicity of phenol at various concentrations in water solution; (2) to determine to what degree toxicity would be affected by the utilization of silica gel as an adsorbent; (3) to determine the effect on growth when mixtures of calcium nitrate and phenol in aqueous solution are used as the medium; (4) to determine the degree of adsorption of phenol in aqueous solution by silica gel.

Methods

As a criterion of toxicity the growth of roots of germinating soy-bean seedlings was used. The salts and organic matter of the seeds in the very early stages of growth are sufficient to prevent starvation.

The methods employed for determining growth rates were essentially the same as those used by TRELEASE and TRELEASE (3) and by EISENMENGER (1). The seeds were germinated on moist filter-paper in glass culture dishes in a dark room.

For each culture a Pyrex beaker of 300-cc. capacity (usual, or tall form without spout) was used. Over the top of this beaker was stretched a piece of paraffined mosquito netting which was secured below the rim by a ligature of paraffined linen thread. This beaker was then placed in a second Pyrex beaker of 600-cc. capacity and the culture solution was poured in until the liquid levels inside and outside the smaller beaker were even at its top.

When the primary roots of the seedlings had an average length of about 10 mm., seedlings were placed on the mosquito netting so that the roots dipped into the culture solution. Duplicate cultures, each of twenty-five plants, were used for each experimental solution.

The cultures were kept in a dark room, and the seedlings were allowed to grow until the primary roots of the control culture had acquired a length of about 98 mm., or until these roots had elongated about 88 mm. (98–10).

The length of the primary root of each individual plant was then recorded, and the average length of the roots of each culture was computed. From this length was deducted the average length of the roots when the seedlings were taken from the germinating dish. This difference constituted the average elongation of the culture. The growth data are relative values. Each was obtained by dividing the average elongation of a given culture by the average elongation of the control culture and multiplying by one hundred.

The average time required for the roots of the control solutions to acquire a length of 88 mm. was 90 hours. Four control cultures of 25 seed-

lings each were used. Also two distilled water cultures of 25 plants each were included in each series. The composition of the control solutions was as follows: 0.02 M KH_2PO_4; 0.02 M $Ca(NO_3)_2$ and 0.02 M $MgSO_4$. The average temperature was 21° C.

The total concentration of phenol and of calcium nitrate was 0.006 M for one series of cultures and 0.06 M for the other. The beakers contained the folowing percentages of the total molecular concentration: 0, 2, 5, 15, 30, 50, 70, 85, and 100. In the solutions containing mixtures of phenol and calcium nitrate the following percentage proportions of 0.06 M of each component were used: 0 + 100, 2 + 98, 5 + 95, 30 + 70, 50 + 50, 70 + 30, 85 + 15, and 100 + 0.

When silica gel was used, 3 grams were placed in each beaker (300-cc. capacity) containing the various proportions of phenol. The contents of the beakers containing the phenol and gel were agitated at intervals during the period of growth.

The silica gel was of 200 mesh size. It had previously been washed until the filtrate gave no test for electrolytes.

In order to determine the apparent degree of adsorption, 300 cc. of the various concentrations of phenol—2, 5, 15, 30, 50, 70, 85, 95, and 100 per cent. of 0.06 M phenol in aqueous solution—were placed in a flask. In each of the flasks were placed 3 grams of silica gel for a period of time equal to the time the plants in the other series were growing. As before, the materials were agitated at intervals. The final concentration of the supernatant liquid was determined by adding an excess of standard bromine solution (Koppeschaar solution) to a portion of it, and titrating the excess of bromine with sodium thiosulphate.

Results

Toxicity of phenol

In the experimental data (table I) it will be noted that as the concentration of phenol becomes higher the toxicity increases—the relative growth becomes less. This is indicated by the percentage proportions of 0.006 M and of 0.06 M (figure 1A and 1B). When the concentration is higher than 0.03 M (50 per cent. of 0.06 M) scarcely any growth occurs.

Growth in solutions of phenol to which silica gel was added

An examination of table I shows that solutions of phenol to which silica gel was added afforded better growth in nearly all concentrations than did the same concentrations of phenol without silica gel. This is likely due to the removal of some toxic principle. Since phenol is not highly ionized, the toxicity is not due to any great extent to the hydrogen ion.

TABLE I

GROWTH OF SOY-BEAN ROOTS IN TOXIC SOLUTIONS AND $Ca(NO_3)_2$

Proportions of 0.006 M	Average relative growth of 50 seedlings each			Proportions of 0.06 M	Average relative growth of 50 seedlings each			Molecular proportions of 0.006 M mixtures of $Ca(NO_3)_2$ and C_6H_5OH		Average relative growth of 50 seedlings each
	C_6H_5OH	C_6H_5OH with silica gel	$Ca(NO_3)_2$		C_6H_5OH	C_6H_5OH with silica gel	$Ca(NO_3)_2$	$Ca(NO_3)_2$	C_6H_5OH	
per cent.	*mm.*	*mm.*	*mm.*	*per cent.*	*mm.*	*mm.*	*mm.*	*per cent.*	*per cent.*	*mm.*
2	71.3	85.9	56.5	2	50.2	60.0	82.9	0	100	2.5
5	70.1	88.1	88.6	5	17.8	28.4	94.4	2	98	2.6
15	56.5	71.7	87.5	15	8.2	6.0	93.9	5	95	2.2
30	51.1	56.2	95.0	30	3.6	4.0	76.5	15	85	3.0
50	17.8	27.4	94.4	50	2.0	3.0	60.0	30	70	5.3
70	7.3	7.5	96.1	70	0	0	54.5	50	50	17.3
85	3.8	6.0	98.5	85	0	0	51.5	70	30	60.8
100	2.5	3.3	95.6	100	0	0	39.4	85	15	78.0
								100	0	95.6

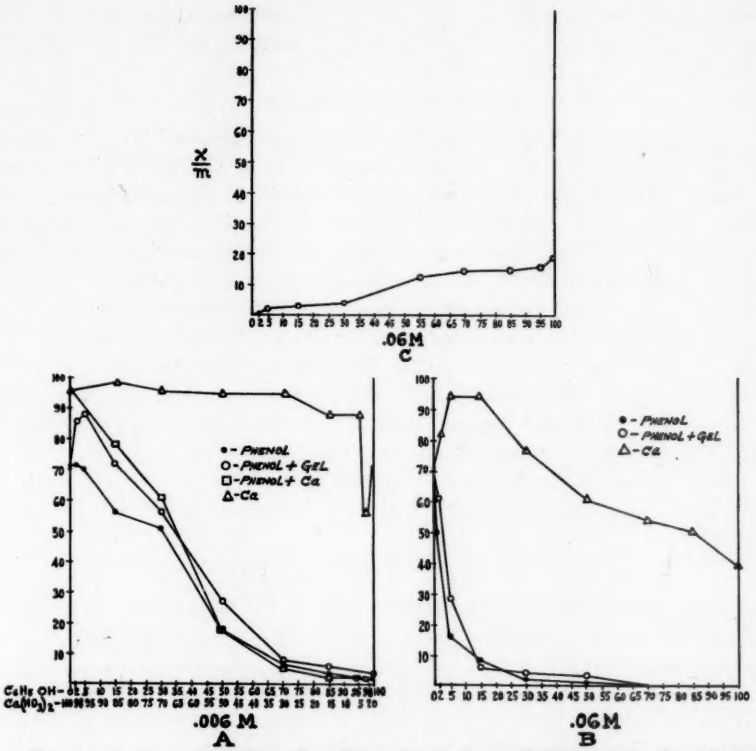

FIG. 1. A. Root elongation of seedlings in solutions of (1) phenol, (2) solutions to which three grams silica gel were added to each phenol solution, (3) solutions containing mixtures of phenol and calcium nitrate, (4) solutions containing calcium nitrate. Ordinates represent percentages of root elongation for standard solution; abscissas represent molecular proportions of 0.006 M. B. Root elongation of seedlings in solutions of (1) phenol, (2) solutions to which three grams of silica gel were added to each phenol solution, (3) solutions of calcium nitrate. Ordinates represent percentages of root elongation for standard solution; abscissas represent molecular proportions of 0.06 M. C. Adsorption of phenol by silica gel. Ordinates represent milligrams of phenol per gram adsorbent; abscissas represent percentage proportions of 0.06 M phenol.

At lower concentrations as in the percentage proportions of 0.006 M the culture solutions containing the silica gel afford an average growth approximately 8 per cent. better than those containing no gel. In the case of percentage proportions of 0.06 M this increased average is slightly more than 4 per cent. As the concentration of the phenol increases, the effectiveness of the gel to detoxify rapidly diminishes. At a concentration of 0.042 M growth is inhibited even though the silica gel is present. In

other words, at low concentrations of a toxic substance the media are relatively more sensitive to modifying agencies of this kind than at high concentrations.

Toxicity of calcium

In the consideration of phenol we could regard the toxicity as the action of the molecule or a part of the molecule. In the case of calcium nitrate we are dealing for the most part with the toxicity of the calcium ion. Soybean seedlings are not decidedly sensitive to this ion; in fact, concentrations lower than 0.03 M seem to be less toxic to the seedlings than is distilled water. The roots of the seedlings grown in distilled water averaged only 71 per cent. of the length of those grown in control solutions. Low concentrations of calcium nitrate may serve to regulate the osmotic pressure which is undoubtedly a factor in distilled water toxicity. It is obvious, however, (figure 1B) that calcium ions are toxic at higher concentrations. When the concentration is equal to 0.042 M (70 per cent. of 0.06 M) there is a decided falling off in growth.

A solution of $Ca(NO_3)_2$ of concentration 0.018 M (30 per cent. of 0.06 M) is about as toxic as one of 0.00012 (2 per cent. of 0.006) solution of phenol.

Toxicity of mixtures of phenol and calcium nitrate

Mixtures of phenol and calcium nitrate throughout all proportions (figure 1A) are more toxic than the corresponding single solutions of calcium nitrate.

When the percentage proportion of phenol exceeds the percentage proportion of calcium nitrate of the 0.006 M mixture, the solution is slightly more toxic than either of the components used singly.

When the percentage molecular proportion of calcium salt exceeds the percentage molecular proportion of phenol, the mixture is less toxic than the corresponding single solution of phenol. The average growth in all the percentage proportions of calcium nitrate is approximately 54 per cent. better than in the corresponding solutions of the mixture.

It seems evident that when phenol has exerted its maximum toxicity no proportions of calcium salt can undo the effects—it has become a type of irreversible reaction. When the normal function of the cells is impaired but not totally disorganized, calcium may serve to remedy the harmful effects to a perceptible degree.

Adsorption

In an attempt to determine quantitatively the degree of adsorption, considerable difficulty was encountered at low concentrations. For the series, 2, 5, 15, 30, 50, 70, 85, 95, and 100 per cent. of 0.06 M phenol solution, values were obtained that seem fairly consistent (figure 1C). For the

same percentage proportions of 0.006 M no consistent increments were obtained by the analytical procedure.

For 300-cc. portions and 3 grams of silica gel with the above percentage proportions of 0.06 M the following values were derived:

M	Grams of phenol per 300 cc. solution	Grams of phenol adsorbed by 3 grams of silica gel
0.0012	0.0338	0.00119
0.0030	0.0846	0.00660
0.0090	0.2540	0.00864
0.0180	0.5080	0.01087
0.0300	0.8467	0.04030
0.0420	1.1854	0.04171
0.0510	1.4394	0.04162
0.0570	1.6087	0.05024
0.0609	1.6934	0.05890

The average adsorption was approximately 3 per cent. of the total amount of phenol present. Silica gel adsorbed approximately 0.03 to 2 per cent. of its weight of phenol.

In view of the fact that adsorption does occur it seems more plausible to attribute the lessened toxic effects to lowered concentration of the media rather than to any oligodynamic phenomena due to retention by the silica gel of substances that could not previously be washed out.

Summary

The toxicity of phenol to plants increases with increased concentration.

Silica gel when placed in aqueous solutions of phenol lowers the degree of toxicity. When the concentration of phenol is increased the detoxifying effect is decreased.

At a total concentration of 0.006 M mixtures of phenol and calcium nitrate exert a toxic effect greater than that of calcium nitrate used singly. When the molecular proportion of calcium nitrate exceeds that of phenol, the toxic effects are less than those of the corresponding single solutions of phenol.

A portion of the phenol was adsorbed by the silica gel.

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STATISTICAL STUDY OF THE TOTAL NITROGEN IN BARTLETT PEAR SHOOTS[1]

A. S. MULAY

(WITH FIVE FIGURES)

When working with biological material, especially trees, it is very difficult to adequately control all the external conditions. Internal conditions, either in plants or animals, are largely beyond control and beyond even such observations as may enable us to select a number of similar individuals. Thus the material to be studied is essentially heterogenous, which makes a statistical study of the material necessary for proper interpretation of the experimental results. The pertinent questions, answers for which are sought in the present study, are: (1) What is the magnitude of the natural variations? (2) How large a population should be taken to get results which will be representative of the group?

Material and methods

The material for this study was collected in the years 1927 and 1928. In 1927, fifty shoots were collected on the 4th of December from 50 uniform five-year-old trees at the University Farm, Davis. In the following year, shoots were collected on the 14th of November from thirty-year-old trees in a commercial orchard.[2] One hundred and fifty shoots were collected at random from a single tree and ninety from as many different trees. Some shoots were also chosen for the color of their bark; forty green and twenty-five brown shoots were collected from a single tree.

All the samples were separated into bark and wood, dried at 50° C., and ground to a fine powder. Another series of 272 shoots collected from a single tree was analyzed for total nitrogen without separating the bark from the wood.

The total nitrogen was determined by the simple Gunning method as described in the official methods of the A. O. A. C. (**1**).

Results

The results are presented as theoretical frequency curves (**3**) in figures 1 to 5, where observed frequencies are marked by circles. Figures 1 and 2 show frequency distribution of the bark and the wood total nitrogen in

[1] I am much indebted to Dr. J. P. BENNETT for his helpful suggestions throughout this work.

[2] This orchard is two miles south of Martinez, California, and has a valley climate. Thanks are due Mr. F. SWETT, owner, for his kindness in furnishing this material.

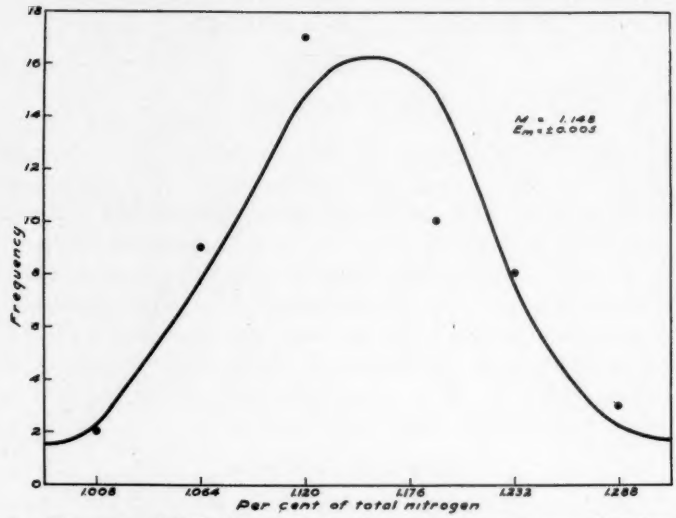

FIG. 1. Frequency distribution of total nitrogen in Bartlett pear 1927 bark from many trees.

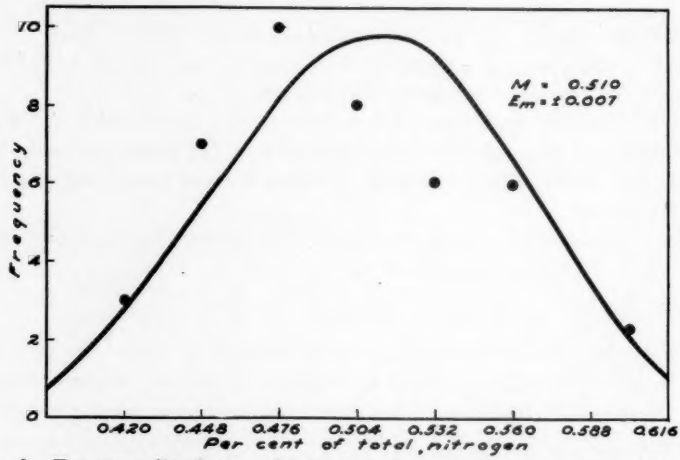

FIG. 2. Frequency distribution of total nitrogen in Bartlett pear 1927 wood from many trees.

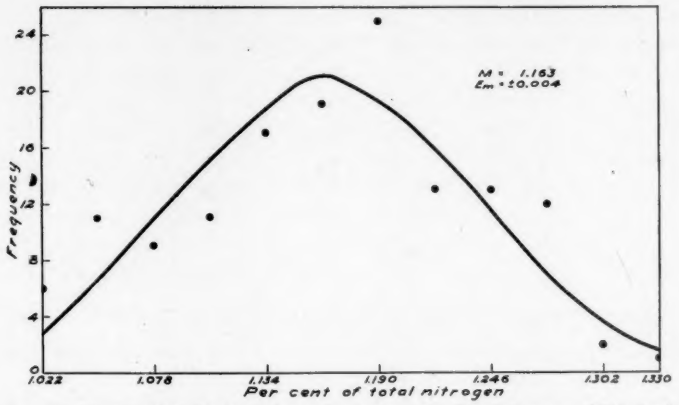

FIG. 3. Frequency distribution of total nitrogen in Bartlett pear 1928 bark from a single tree.

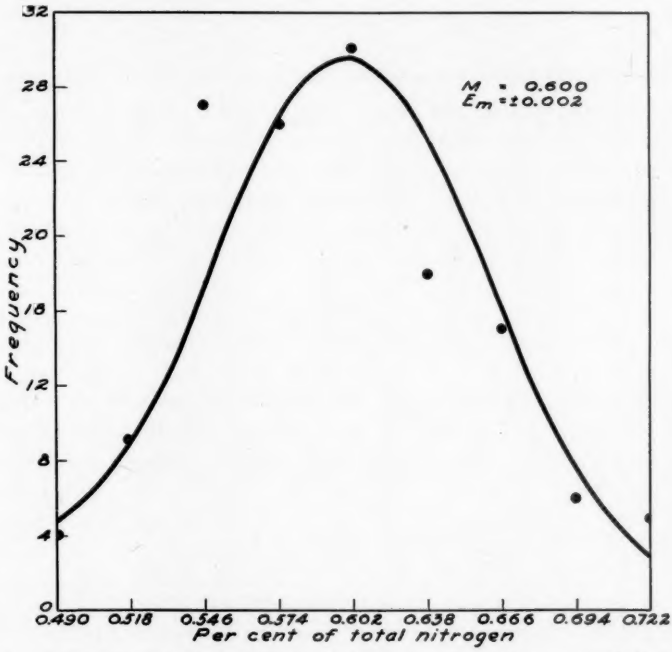

FIG. 4. Frequency distribution of total nitrogen in Bartlett pear 1928 bark from a single tree.

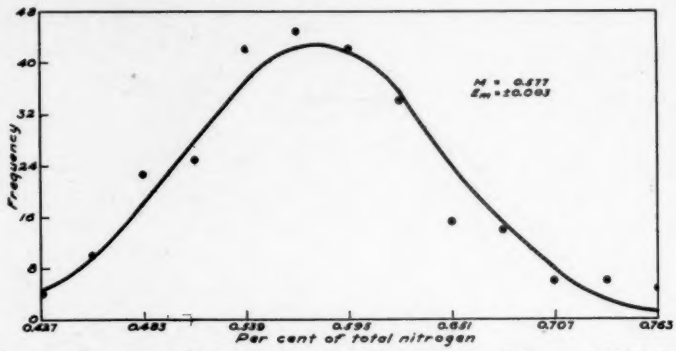

FIG. 5. Frequency distribution of total nitrogen in Bartlett pear 1928 twigs.

shoots from 50 different trees of the 1927 collection. Figures 3 and 4 give the nitrogen distribution in the bark and the wood from shoots collected at

TABLE I

NUMBER OF VARIANTS, MEAN, QUARTILE, AND NUMBER REQUIRED FOR DESIRED ASSURANCE, FOR THE DIFFERENT SERIES IN THE VARIABILITY STUDY

		SERIES	NUMBER OF VARIANTS	MEAN	QUARTILE	NUMBER REQUIRED FOR DESIRED ASSURANCE
From different trees	Davis 1927	Bark	49	1.148 ± 0.007	0.0456	10
		Wood	48	0.510 ± 0.005	0.0375	35
	Commercial orchard, 1928	Bark	87	1.225 ± 0.008	0.0440	8
		Wood	87	0.651 ± 0.006	0.0321	15
From one tree		Bark	149	1.163 ± 0.004	0.0498	12
		Green	40	1.077 ± 0.005	0.0304	5
		Brown	25	1.244 ± 0.004	0.0220	2
		Wood	149	0.600 ± 0.002	0.0349	22
		Green	40	0.585 ± 0.004	0.0244	11
		Brown	25	0.652 ± 0.005	0.0239	8
		Twigs	272	0.577 ± 0.003	0.0475	44

random from a single tree in 1928. Figure 5 shows nitrogen distribution of unseparated shoots from a single tree. As the results for the wood and the bark of the shoots collected from many trees in 1928 are similar to those for the shoots of 1927 they are not presented here.

Table I gives the number of variants, the mean, the quartile or P. E. Single (Q), and the number of shoots (N) required to assure the odds of 142:1 that the true value lies within $\pm$ 5 per cent. of the observed value. The formulae used in this connection were:

$$Q = 0.6745 \sqrt{\frac{\Sigma fd^2}{n-1}}$$

$$N = \frac{(\text{Coefficient of odds} \times \text{P. E. Single})^{(2)}}{\text{Deviation}}$$

Discussion

The results show that there is considerable difference in the extremes of the total nitrogen content both in the bark and in the wood of the pear shoots collected at the same time either from one or from many trees. Figures 1 and 2 for shoots from Davis show that the highest total nitrogen value for the wood is about 150 per cent. of the lowest, and the highest for the bark about 134 per cent. of the lowest. The bark of the shoots collected from a single tree shows differences (figure 4) similar to those in the bark of shoots from many trees, while the highest value for the wood from the shoots collected from a single tree (figure 3) is only about 136 per cent. of the lowest. The differences in the extreme values is much reduced both in the bark and in the wood when the shoots are selected by color. In the unseparated shoots this difference is about 80 per cent. of the lowest.

From table I it is seen that the bark and the wood of the green shoots have a smaller mean nitrogen content than the bark and the wood of the brown shoots. This shows that the total nitrogen content of the shoots varies according to the physiological age of the shoots and gives some ground for thinking that the differences in the total nitrogen content of the shoots collected at the same time may in part be due to the differences in their physiological age, though they may not show any visible evidence of this age.

Comparison of the quartiles in table I shows that the bark and the wood samples of 1927 are very similar to those of 1928 and in the 1928 samples those from many trees differ very little from those from one tree. The shoots collected from many trees are not more variable than those from one tree. The bark in general seems to be more variable than the wood, the quartile for bark being about 0.046, and for wood about 0.035. The shoots selected by color are less variable than those collected at random.

The quartiles for the green and the brown bark are 0.030 and 0.022 and those for wood from the green and the brown shoots are 0.024 and 0.024.

The last column in table I gives the number of shoots required to assure that the total nitrogen values observed are within ± 5 per cent. of the true value with the odds of 142:1. The number of the shoots required for this assurance is 8 to 12 for the bark, while for the wood it is 22 to 35. About 35 shoots from Davis pear trees are required to get a representative wood sample. As these values are not very precise, it is desirable, that whenever possible 40 or more shoots should be taken for a sample to assure a good representation.

Summary

Individual shoots either from the same or from different trees vary considerably in their total nitrogen content. The highest value for bark is 35 per cent. and for wood 50 per cent. higher than the respective lowest values. The shoots collected in 1927 are similar in their variability to those collected in 1928. The shoots collected from different trees do not vary any more than those collected from a single tree.

The bark and the wood from the green shoots are lower in their total nitrogen content than that from the brown shoots. This shows that the differences in the physiological conditions of the shoots may in part explain the variations in their total nitrogen content.

A sample of about 35 to 40 shoots is required to assure the odds of 142:1 that the true value lies within ± 5 per cent. of the observed value.

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COPPER AS AN ESSENTIAL FOR PLANT GROWTH[1]

A. L. SOMMER

(WITH THREE FIGURES)

The stimulating effect of copper on plant growth was noted early in the use of copper salts as fungicides. A few years ago FELIX (**3**) obtained improvement in the growth of certain plants on several peat soils by the application of copper sulphate, both to the soil and in solution to the leaves. ALLISON, BRYAN, and HUNTER (**1**) were able, by the use of copper sulphate, to produce crops on certain otherwise unproductive peats of the Florida Everglades. Other treatments, notably caustic lime, manganese sulphate, and manure also gave improvement, but were not so beneficial as copper sulphate. BRYAN (**2**) also obtained greening in chlorotic leaves of plants grown in this soil by treating them with solutions of copper sulphate or manganese sulphate. These investigations do not furnish final proof, however, that copper is essential to plant growth. The work reported in this paper provides additional evidence on this point.

Sunflowers, tomatoes, and flax were used in these investigations. One-liter pyrex beakers with paraffine-coated, plaster of Paris covers were used as containers for the solutions in which the plants were grown. All water used in making up the nutrient solutions was redistilled from pyrex. In the first experiment with sunflowers the salts used had been repurified for an earlier study (**5**) on the effects of the absence of boron on plant growth. These salts had been recrystallized from water from a copper still; this still had the usual block tin condenser. In later experiments the water used for the purification of the salts was redistilled from pyrex. The methods used for the repurification of the salts[2] are described in a previous paper and will not be reiterated here.

The solutions to which copper was or was not added had the following composition:

[1] Presented before the Division of Biological Chemistry at the 78th meeting of the American Chemical Society, Minneapolis, Minn., Sept. 9–13, 1929.

[2] Analyses made recently in the laboratory of Professor FRED ALLISON (see Journal of the American Chemical Society **52**: 3796–3806. 1930, for method) on samples of some of these salts showed that copper in a concentration of about 5×10^{-7} was present in the solutions because of copper added as an impurity of the KNO_3, KH_2PO_4, and $MgSO_4$. Unfortunately samples of the other salts and the distilled water used were not available so that the total copper concentration of solutions to which copper was not intentionally added could not be determined. The amount added as impurities in the salts must, however, have been very small.

	per liter		per liter
KNO_3	0.80 gm.	$MgSO_4 \cdot 7H_2O$	0.50 gm.
KH_2PO_4	0.15 "	$CaSO_4$, saturated solution,	300 cc.

Iron was added in the form of $FeSO_4$ as the plants needed it. In the work with sunflowers, traces of the following elements were also added: manganese, aluminum, iodine, fluorine, sodium, chlorine, and boron. In addition to these, traces of tin, rubidium, lithium, barium, mercury, nickel, cobalt, arsenic, and lead were added to the cultures of tomatoes and flax. An excess of SiO_2 was added in all cases.

Dwarf sunflowers were used as experimental plants in the first investigation. The cultures were divided into two groups, one with and one without copper. Five cultures of two plants each were included in each group. Copper, as copper sulphate, was added to the solutions receiving copper at the rate of 0.125 mg. per liter. Two subsequent additions, one of 0.125 mg. and one of 0.06 mg. were made.

FIG. 1. Sunflowers grown with and without copper. Upper row with copper; lower row without copper.

The plants were transferred to the experimental solutions while in the cotyledon stage. By the end of the week, the plants in solutions containing copper began to show better growth than those without copper. Although very variable in size, those receiving copper appeared normal, and all were blooming at the time of harvest. Only one plant without copper produced a bud which was very small and appeared abnormal. The average dry weight per plant of those receiving copper was 4.2 gm.; that of the plants without copper was 0.31 gm. Plants grown with and without copper are shown in figure 1.

Sunflowers of a second series did not do so well. The reason for the poorer growth was not determined. Conditions which might have been causative, however, were: (1) the culture solutions became overheated when the temperature control of the conservatory was out of order, and (2) the salts used in the preparation of the nutrient solution were repurified from water redistilled from pyrex; consequently they may have been free from impurities which may have been beneficial to the plants of the preceding series. With the exception of one plant, those without copper were noticeably poorer within three weeks than those with copper. This plant appeared similar to the control plants for several weeks, and was as large as some of them when harvested. About the time it produced a bud, a little later than most of the control plants, it began to appear to be a very sick plant, and the bud did not develop. The average dry weight of plants without copper was 0.16 gm. Four plants were dead and the rest were in very poor condition. The average weight of plants with copper was 0.70 gm. The tops of these plants appeared normal, but the roots, like those of the plants without copper, were badly infected with fungi. Copper was added in this experiment at the rate of 0.06 mg. per liter. Four additions were made during the experiment.

Tomatoes (Dwarf Champion) were used as experimental plants in a subsequent investigation. This series was divided into two groups of six cultures each, one group with and one without copper. There were three plants to each culture. A single addition of 0.06 mg. copper per liter of solution was made to each of the six cultures with copper.

The plants were transferred to the experimental solutions in the cotyledon stage. All plants grew well for the first week. Soon after this, some of the plants without copper began to appear sickly; two were dead by the end of the second week; by the end of the third week a third plant had died. All plants with copper made good growth until the beginning of the seventh week, when one of the plants wilted; this plant was in very poor condition when the plants were harvested at the beginning of the ninth week. Most of the plants with copper had buds, and all except the one mentioned were in excellent condition. The average green weight

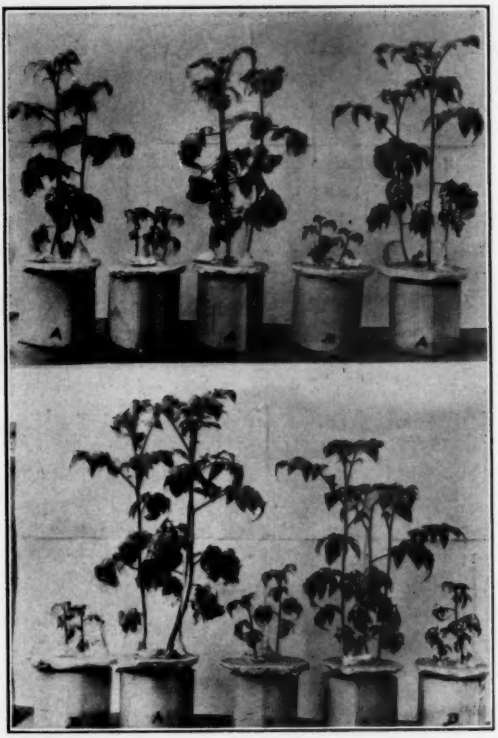

FIG. 2. Tomatoes grown with and without copper. Cultures (A) grown with copper; cultures marked (B) grown without. Only 0.06 mg. copper per culture was used.

of plants without copper was 2.9 gm.; that of plants with copper was 31.3 gm. The average dry weight of plants without copper was 0.3 gm., while that of plants with copper was 2.6 gm. The average did not include the three plants without copper which had died nor the one with copper which was badly wilted. The plants at time of harvest are shown in figure 2.

It is well known that copper is toxic to green plants even in relatively low concentrations. It was found by the writer in earlier work that there was considerable inhibition of root development for some plants at a concentration of 0.25 mg. per liter. It is, therefore, not surprising that a single addition of 0.06 mg. of copper would determine whether three plants would produce, as the largest culture did, 142.2 grams of green matter as compared with 12.8 grams, the weight of the largest culture without copper.

Moreover, it remains to be determined how small an amount of copper will be sufficient to produce an adult tomato plant.

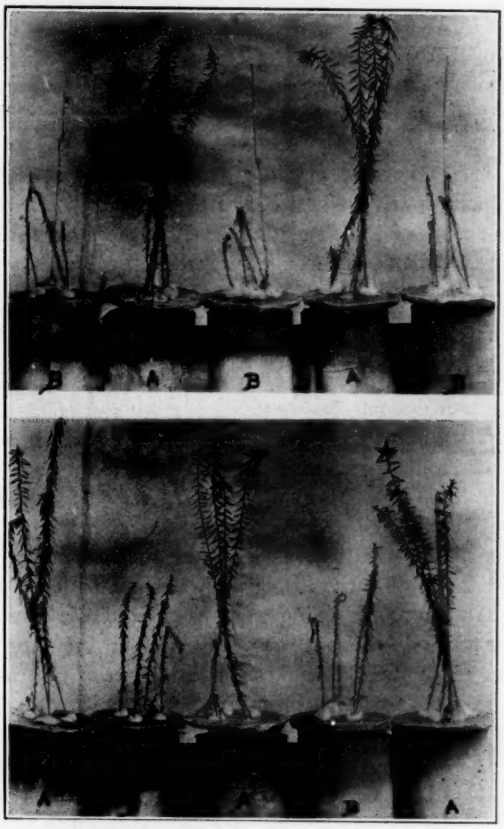

FIG. 3. Flax grown with and without copper. Cultures (A) grown with copper, cultures (B) without.

Flax was the third plant investigated. As in the previous experiments the plants were transferred in the cotyledon stage to the solutions to which copper had or had not been added. All plants grew well for the first week. By the middle of the second week the plants without copper were noticeably smaller than those with copper. About the end of the third week the roots of all plants appeared somewhat abnormal, and the plants with copper seemed to be growing more slowly than before. A second addition of 0.06

mg. of copper was made to each of the cultures with copper. Because this addition apparently was of no benefit, an addition of traces of all elements previously used in very small amounts was made to all cultures (cultures without copper as well as those with copper) a week later. The plants with copper began to show improvement within a few days, but those without it appeared to make no further growth. Six of the plants were dead at the time of harvest, fifty-two days after they were transferred to the experimental solutions. Most of the plants with copper had buds at this time but were not of normal size, as were plants grown at the same time in solutions of salts of ordinary purity. This indicates that flax has a larger requirement for one or more of the elements added in traces than does the tomato plant. The dry weight of eighteen plants without copper was 1.4 gm.; that of the same number of plants with copper was 4.5 gm. Plants grown with and without copper are shown in figure 3.

The investigations of FELIX, of ALLISON, BRYAN, and HUNTER and of BRYAN, even though showing very beneficial effects of applications of copper, did not furnish conclusive proof that this elements is essential to plant growth. SMITH (**4**) has shown that there is a toxin in the black moor soils (Gliede) of Holland which prevents normal plant growth, and which is rendered non-toxic by copper sulphate. It may be, therefore, that the beneficial effects obtained by the above mentioned investigators were due to a similar chemical action in the soils when the reagents were applied to the soils, or in the leaves when they were treated with solutions of these reagents. BRYAN showed greening of spots on chlorotic leaves where solutions of copper sulphate or manganese sulphate had been applied. A manganese deficiency is known to produce a certain type of chlorosis, but plants which were grown in solutions of purified salts to which no copper had been added were never chlorotic at any stage in their growth or decline. We have as yet no clew as to what the rôle of copper in plant metabolism may be, but the idea that it may act as an autooxidant is intriguing since it is well known that an extremely small trace of this element will act as a catalyst, greatly hastening the process of rancidity in fats. This catalytic property may also explain why both copper and manganese salts are so beneficial when applied to certain organic soils.

I wish to express my appreciation for the aid and encouragement which I received from the late Professor J. ARTHUR HARRIS in this work during my sojourn at the University of Minnesota.

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RELATION OF CATALASE ACTIVITY TO PHYSIOLOGICAL BREAKDOWN IN JONATHAN APPLES[1]

J. R. NELLER

(WITH TWO FIGURES)

Considerable attention is being given to premature breakdown in apples for the reason that the disease constitutes a problem of both scientific and economic interest. This breakdown, which is physiological in nature, occurs in several varieties and has been described by several investigators (**2, 3, 6, 10, 11, 12**).

In the Pacific Northwest the Jonathan variety of apple is particularly susceptible to breakdown, which appears to be associated with amount of fruit per tree and the condition of the tree. However, the fundamental causes are still largely unknown.

If catalase activity may be considered as an index of the growth or nutritive condition of a plant as suggested by HEINICKE (**4**), and again by KNOTT (**7**), it may also be an index of the metabolic rate of activity in the flesh of an apple that is passing through a state of physiological breakdown.

Method of determination

An apparatus (figure 1) was designed that was somewhat different than that described by APPLEMAN (**1**). A water bath contained a wide mouthed reaction bottle of 60-cc. capacity; a three-hole rubber stopper fitted into this bottle, one hole being for the stirring mechanism, one for the ingoing hydrogen peroxide, and one for the outgoing oxygen. The glass stirrer was driven by a motor equipped with a variable speed reducer and was lubricated in its contact with the rubber stopper with a mixture of powdered graphite and glycerin. The hole in the stopper was cut larger than the glass stirring rod to enable a piece of rubber tubing to cover the rod where it passed through the stopper. It was found that a gas tight bearing was thus obtained that could easily be kept lubricated with the graphite glycerin mixture. As shown in figure 1 a hydrogen peroxide chamber, consisting of the bulb part of a 10-cc. pipette, was built into the system using two U-shaped glass T's. This enabled the operator to allow the hydrogen peroxide to mix with the catalase suspension after the entire system had been closed and the stirring started without changing the initial gas volume of the apparatus. During the course of the reaction

[1] Published with the approval of the Director of the Washington Agricultural Experiment Station as Scientific Paper no. 173, College of Agriculture and Experiment Station, State College of Washington.

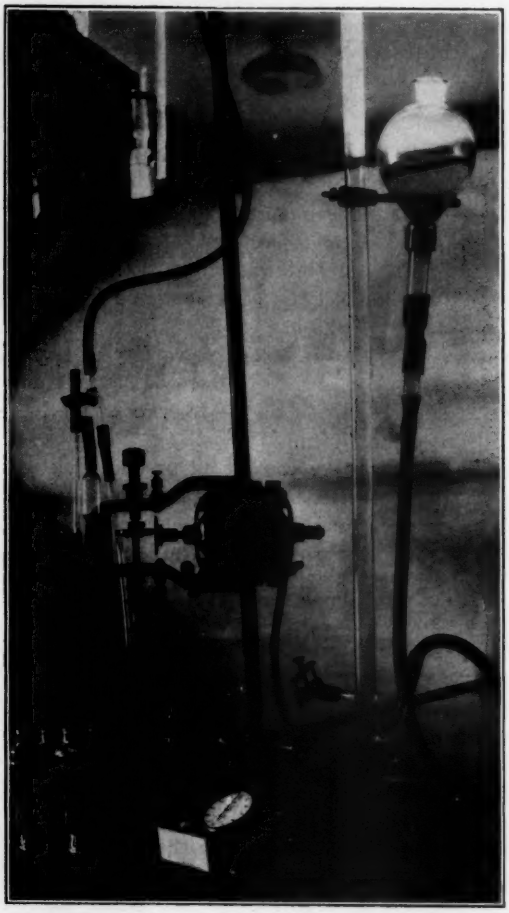

FIG. 1. Apparatus used to determine catalase activity.

the leveling bulb was lowered occasionally so as to maintain an approximate pressure within the system. Readings were taken from the gas burette at one minute intervals with the use of a stop watch. All determinations were made at a temperature of 20° C. At first a record of air temperatures and barometric pressures was kept, but this was not continued as it was found that the greatest correction of the gas volumes thus obtained was considerably less than the variation between samples.

The work of various investigators has shown that the usual method of determining catalase activity by measuring the ability of the enzyme to decompose hydrogen peroxide results in an activity measurement of a relative value only, depending upon the conditions of measurement, such as method of preparing the sample, temperature of reaction, and H^+ ion concentration. Thus HEINICKE (5) found that it was necessary to neutralize tissues with low pH values before consistent catalase values could be obtained. OVERHOLSER (9) neutralized his pear tissue with calcium carbonate and found that what he terms the quantity of catalase obtained thereby was greater but in the same order as the catalase activity of a like set of unneutralized samples. The pH of his calcium carbonate treated samples ranged from 6.4 to 7.0.

In the present work it was decided to make all of the determinations in the presence of an excess of calcium carbonate. Samples were first prepared by using immediately a portion of the pulp obtained by passing the apples through a grater and then grinding in a mortar with calcium carbonate and quartz sand. It was found that the catalase activity was much lower than when portions of the same apples were ground in a mortar without passing through a grater. It was also found that there was much more catalase in the peel than in the flesh and very little in the expressed juice. Accordingly the following procedure was adopted. Apples were cut in halves (three to ten fruits per sample) with the line of cutting through the cheek and green side of the fruit. Thin triangular slices were then cut from the halves, care being taken to include the proper proportion of skin and core. A sample of twenty grams was macerated with five grams of precipitated calcium carbonate, adding enough water and quartz sand to facilitate grinding to a smooth paste. This was made up to 100 cc. and duplicate 20-cc. portions were pipetted into the reaction bottles which were placed in the water bath held at 20° C. The hydrogen peroxide was also held at a temperature of 20° C. in the bath. At first it was shaken with calcium carbonate before using, but it was found that this was unnecessary as no gas was given off thereby. After inserting the rubber stopper and stirrer the motor was started and the gas burette evacuated. The system was closed and kept under a vacuum of three or four inches of water for a half minute to make sure that there were no leaks. The 10 cc. of 3 per cent. hydrogen peroxide already introduced into the system were allowed to flow down into the catalase suspension and the stop watch was started. Gas volume readings were then taken every minute for four minutes or longer. Except for the July 2 determinations (table III) not more than 70 of the 100 cc. of available oxygen were needed to effect a cessation of gas liberation.

Experimental

The apples used in these experiments were grown in the Wenatchee Valley[2] and were put into cold storage at a temperature of about – 1° C. as soon as harvested. Upon removal from cold storage they were immediately subjected to catalase determinations and analysis.

During the winter of 1928 preliminary experiments with fruit of the 1927 harvest established the standard procedure that was adopted as described above. In March of 1929 some catalase determinations were made on two lots of Jonathans of the 1928 harvest, one of which did and the other did not display the characteristic physiological breakdown. These show (table I) that catalase activity tended to be higher in the fruit that was breaking down.

TABLE I

RELATIVE CATALASE ACTIVITY OF NORMAL AND ABNORMAL LOTS OF JONATHAN APPLES OF 1928 HARVEST

SAMPLE	LOT 1*, SHOWING NO BREAKDOWN	LOT 2*, SHOWING BREAKDOWN	HALVES SEALED TO GLASS PLATES FOR 10 DAYS AT ROOM TEMPERATURE	
			FROM LOT 1	FROM LOT 2
	cc.	*cc.*	*cc.*	*cc.*
1	35.7	57.1		
2	35.1	39.7		23.0
3	46.5	68.5	67.8	

* Determined fresh from cold storage in March, 1929. Given in cc. of O_2 per 4 gm. tissue at 20° C.

Certain of the halves of the apples used for these determinations were sealed to glass plates with paraffin and held at room temperature for 10 days when they were analyzed for catalase activity. As shown in table I the halves of the sound apples of sample 3 had catalase values of 46.5 and 67.8 from cold storage and after 10 days at room storage, respectively, while corresponding values for the broken down apples of sample 2 were 39.7 and 23.0. Thus the catalase activity of the sound fruit, starting low, tended to go up and that of the broken down fruit, starting high, tended to go down when stored at an elevated temperature. This result supports the theory that physiological breakdown of Jonathan apples is associated with a high metabolic rate which later falls off below that of sound apples.

[2] The samples were kindly supplied by Prof. F. L. OVERLEY of this Station in connection with his production and storage studies.

An analysis (table II) of these apples as taken fresh from cold storage indicates that those in the process of breaking down tended to be somewhat higher in dry matter, sucrose, and nitrogen.

In a further study of the course of catalase activity some sound apples were subjected to catalase determinations when fresh from cold storage and after 26 and 43 days at room temperatures. Figure 2 shows that whereas

TABLE II

COMPOSITION OF JONATHAN APPLES FRESH FROM COLD STORAGE IN MARCH, 1929

SAMPLE AND CONDITION	TOTAL SUGAR	INVERT SUGAR	SUCROSE	ACID $\frac{N}{10}$ FOR 5 GM.	NITROGEN	DRY MATTER
	per cent.	*per cent.*	*per cent.*	*cc.*	*per cent.*	*per cent.*
Halves of breakdown lot showing least breakdown	10.69	8.76	1.93	2.6	0.048	15.81
Remaining halves of above, showing breakdown	11.02	8.45	2.57	2.2	0.046	15.98
Sound apples of above breakdown lot	10.58	8.90	1.68	3.2	0.049	15.34
Lot without visible breakdown	10.34	9.04	1.30	2.7	0.033	14.69

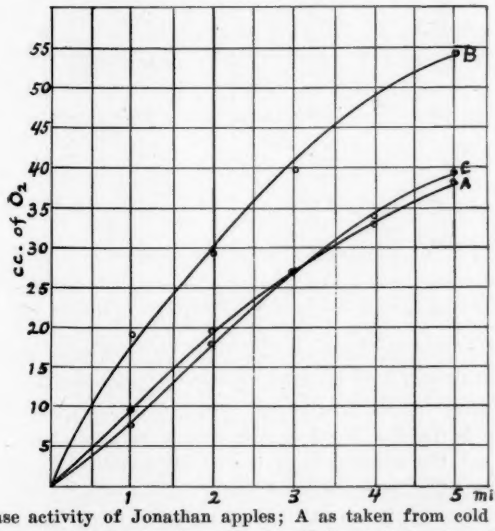

FIG. 2. Catalase activity of Jonathan apples; A as taken from cold storage; B after 26 days at room temperature; C after 43 days at room temperature.

the activity rate was considerably higher at the end of 26 days, it had dropped slightly below the initial rate at the end of 43 days. This rise and fall of catalase activity during the youth and senescence of the apples was probably more pronounced than the graph indicates, as gram equivalents were used at all dates and those after 43 days of room storage contained more dry matter than those fresh from cold storage. In this connection it is of interest to note that MAGNESS and BURROUGHS (**8**) found with an 18° C. storage that the catalase activity of apples that had been previously stored at 0° C. was higher than that of fruit previously stored at 1.7° C. OVERHOLSER (**9**) working with pears, has reported that prolonged storage at 20° C. caused the catalase activity to be much reduced over that of fruit stored at 0° C.

These findings caused an extension of the catalase determinations that were made on the next year's crop to include apples in advanced stages of breakdown. On five different dates (table III) samples of normal, broken

TABLE III

RELATIVE CATALASE ACTIVITY OF JONATHAN APPLES OF 1929 HARVEST FROM A LOT CONTAINING SOUND FRUITS AND FRUITS IN VARIOUS STAGES OF BREAKDOWN.*

DATE	SOUND APPLES	BREAKDOWN SHOWING	ADVANCED STAGES OF BREAKDOWN
	cc.	*cc.*	*cc.*
March 14	28.6	44.9	
April 8	32.1	40.0	27.2
April 30	33.3	46.7	12.5
May 15	40.6	57.8	11.1
July 2	68.9	91.7	13.5
Average	40.7	56.2	16.1

* Expressed in cc. of O_2 obtained in 4 min. from 4 gm. of tissue.

down, and badly broken down fruit were taken from cold storage for catalase determinations. As found in previous years catalase activity was considerably higher in the fruits that were showing breakdown. It was distinctly lower in fruits in advanced stages of breakdown. It may be observed also that the catalase activity of all samples tended to increase with increasing periods of storage with the exception of fruits in advanced stages of breakdown, which tended to decrease.

Summary and conclusions

A convenient type of apparatus for catalase determinations is described and a standard method of procedure is outlined.

As a result of a study extending over three seasons on the catalase activity of Jonathan apples as related to physiological breakdown, it was found that catalase activity tended to be higher in apples going through the breakdown process and to be decreased, below that of normal fruits, in fruits in advanced stages of breakdown. It was found also that the catalase activity of apples that did not develop breakdown tended to increase during the earlier and to decrease during the later periods of storage corresponding to the youth and senescence of the apples. These findings corroborate the theory that catalase activity measurements may be used as an index of the rate of metabolic activity. The data lead to the opinion that physiological breakdown in apples is associated with or caused by an accelerated metabolic rate.

The fundamental causes of physiological breakdown are still somewhat obscure. It is known, however, that the disease is most liable to occur when the crop on a tree is fairly small and the fruits correspondingly large (**2**).[3] This is in line with the general tendency of fruit showing breakdown to be higher in per cent. of dry matter and sucrose.

If the breakdown is caused by an accelerated rate of metabolic activity a search for the cause might well include a study of physiological balance with regards to the food supply of the tree and its fruits. It is possible that more attention should be given to pruning and thinning operations so as to insure an optimum fruit load per tree. The complexity of interrelated factors that influence apple trees grown under intensive cultural conditions causes the study of physiological breakdown to be a difficult problem.

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IS ELECTRODIALYSIS USEFUL IN A STUDY OF APPLE TISSUE[1]

J. R. NELLER

(WITH ONE FIGURE)

The separation of ions from colloids by the process of electrodialysis is a procedure that is being used with many types of materials. It has been especially favored since the introduction of the three compartment cell described by MATTSON (**1**).

MOORE, REEVES, and HIXON (**2**) appear to have been the first to employ this electrolytic method of dialyzing fruit tissue. They used the peels from Jonathan apples and found that samples from fruits affected with "Jonathan spot" reacted differently than those from normal fruits.

Experimental

During the course of a study of the cause of Jonathan breakdown (**3**) some samples were electrodialyzed in an endeavor to learn how the normal and abnormal apples might differ from each other. A three compartment cell was made much like the one described by MOORE, REEVES, and HIXON (**2**) except that instead of moulding a set of rubber sections, they were obtained by sawing a hard rubber battery box into three parts (fig. 1).

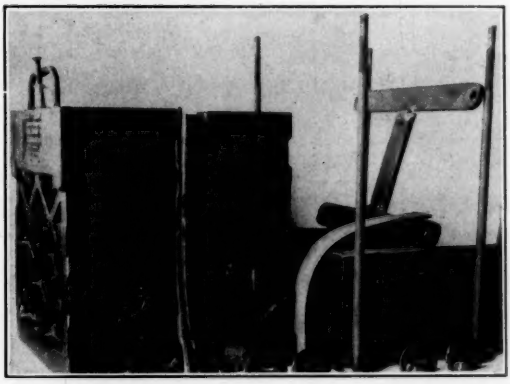

FIG. 1. Three-compartment electrodialysis cell, made from a hard-rubber battery box, with the rubber gaskets and clamping device.

[1] Published with the approval of the Director of the Washington Agricultural Experiment Station as Scientific Paper no. 174, College of Agriculture and Experiment Station, State College of Washington.

Four rubber gaskets were cut to fit the sawed sections of the box and one was put on each side of the parchment paper membranes. The cell became water tight when clamped together with four bolts.

Three hundred grams of apple tissue were put through a grater and suspended in 750 cc. of distilled water in the central compartment. A similar amount of water was placed in the cathode and anode compartments into which platinum coil electrodes were inserted. A potential of 110 volts was maintained for six hours, at the end of which time the cathode and anode solutions were drained from the cells and replaced with distilled water.

Temperatures taken in the central compartment at the end of each of the three six-hour runs per sample were found to average 62°, 47° and 40° C., respectively, and the corresponding current readings averaged 800, 630 and 275 milliamperes. At the beginning of each run the temperatures averaged 23° C. and there was a current flow of only a few milliamperes.

Table I shows that there was but little variation in the hydrogen ion concentration of the three extractions removed from the anode compart-

TABLE I

H-ION CONCENTRATIONS OF SOLUTIONS IN CATHODE AND ANODE CHAMBERS IN THE ELECTRODIALYSIS OF JONATHAN APPLE TISSUE

TYPE OF SAMPLE	ANODE COMPARTMENT			CATHODE COMPARTMENT		
	RUN 1	RUN 2	RUN 3	RUN 1	RUN 2	RUN 3
	pH	*pH*	*pH*	*pH*	*pH*	*pH*
Small apples, lot 1,* no breakdown present	3.10	2.88	3.35	Over 8	4.95x	7.0
Large apples, lot 2, no breakdown showing	2.80	3.13	3.30	Over 8	6.86	7.0
Apples of above lot 2, with breakdown showing	2.83	2.88	3.34	Over 8	7.75	6.88

* Lot 1 and lot 2 apples averaged 115 and 180 grams respectively. Each run was of six hours' duration.

x This solution stood over night in the cell with current turned off.

ment. In the cathode chamber the first extract was alkaline, while the second and third were practically neutral. pH values greater than 8.0 are not recorded for the reason that a quinhydrone electrode was used.

It may be observed (table I) that there was little difference in the hydrogen ion concentrations of extracts from normal and abnormal apples. These apples were distinctly different in that the normal lot 1, *viz.*, showing

no breakdown, were quite small, averaging only 115 grams per apple, having been unable to grow larger because of a restricted leaf area control,[2] while those of lot 2, were extra large and averaged 180 grams per apple.

TABLE II

ACID AND BASIC MATERIALS EXTRACTED BY THE ELECTRODIALYSIS OF JONATHAN APPLES EXPRESSED AS CC. OF N/10 SOLUTIONS

TYPE OF SAMPLE	ACIDITY, ANODE COMPARTMENT				ALKALINITY, CATHODE COMPARTMENT			
	RUN 1	RUN 2	RUN 3	TOTAL	RUN 1	RUN 2	RUN 3	TOTAL
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
Small apples, lot 1, no breakdown present	55.0	61.0	14.0	130.0	110.0	6.0	None	116.0
Large apples, lot 2, no breakdown showing	80.0	40.0	43.0	163.0	96.0	1.0	None	97.0
Large apples, lot 2, with breakdown showing	67.0	58.0	18.0	143.0	104.0	4.0	None	108.0

Neither were there any distinctive differences in the total amounts of acid and basic material extracted from these samples (table II). Somewhat more acid, however, was the extract from the normal apples of lot 2, but this was probably due to the fact that the tissues contained about 40 per cent. more total acid than those of the apples of lot 1.

An analysis of these extracts for NO_3, Cl, SO_4, K, and Ca ions showed (table III) that the only one of these ions that was extracted in any appreciable amount was potassium. Sodium and malic acid were not determined but must have been present in large amounts.

Since the ionic constituents of apple tissue consist largely of malic acid and its salts, a study was made of the dialysis of malic acid. Sugar and basic material were added to make the mixture more like that of apple tissue. This mixture consisted of 60 cc. of N/1 malic acid, 11 cc. of N/1 sodium hydroxide, and 30 gm. of grape sugar, the whole being diluted to 750 cc. These ingredients approximate those of the samples of apples discussed above.

After a six-hour dialysis of this mixture the values given in table IV were obtained. The acidity accumulation in the anode solution was higher than from the apple tissue samples due possibly to the presence of less adsorbing material in the synthetic sample. But since the pH value of the anode solution was considerably lower than that of a pure malic acid

[2] Dr. J. R. MAGNESS and Prof. F. L. OVERLEY kindly furnished these samples from their leaf area experiments.

TABLE III

IONIC CONCENTRATIONS FOUND IN EXTRACTS DIALYZED FROM APPLE TISSUE EXPRESSED AS PERCENTAGE OF WEIGHT OF TISSUE USED

TYPE OF SAMPLE	NO_3	Cl	SO_4	K AS K_2O	Ca AS CaO	TOTAL ASH
		per cent.	*per cent.*	*per cent.*		*per cent.*
Small apples, no breakdown	Trace	0.0032	Trace	0.19	Trace	0.279
Large apples, no breakdown showing	Trace	0.0015	0.0016	0.16	"	0.320
Large apples, broken down fruits of above lot	Trace	0.0026	0.0020	0.17	"	

solution of the same concentration which was found to have a pH of 2.65, it is believed that some active acidity was introduced as an impurity in the dextrose that was used.

TABLE IV

ELECTRODIALYSIS OF A MALIC ACID-DEXTROSE-SODIUM HYDROXIDE SOLUTION MADE UP TO SIMULATE APPLE TISSUE FLUID

TOTAL ACIDITY IN ANODE CELL	TOTAL ALKALINITY IN CATHODE CELL	pH OF ANODE SOLUTION	CENTRAL CELL TEMPERATURE	CURRENT VALUES		pH OF RESIDUE IN CENTRAL CELL
cc. N/10	*cc.* N/10	*pH*	° *C.*	*milli-amperes*	*volts*	*pH*
151	44	2.03	55	800	100	2.70

At any rate, the results show that malic acid dialyzed fairly rapidly under the conditions imposed. The analyses given in table III, indicate that it accounted almost entirely for the acidity obtained in the apple tissue dialysis. Since there is so much malic acid in apple tissue, it seems that its progressive dialysis would mask any possible difference as to the amount, or manner in which it might be held, in normal and abnormal tissue. This would explain the similarity in acid extractions obtained from the normal and broken down fruits of the above experiment (table II). In this connection it is to be recalled that MOORE, REEVES, and HIXON obtained differences (**2**) in the rate of acid dialysis from normal apple peels and from those affected with Jonathan spot.

As found by these authors, the basic material that was obtained was removed during the early stages of dialysis (table II). While about the

same per cent. of malic acid was extracted from their samples, as in the present case, the percentage of basic material that they obtained was considerably higher. This suggests that much of the cation material of an apple is located in or near the skin. Table II, shows that about the same amount of basic material was obtained from normal apples as from those that were in stages of physiological breakdown.

These data tend to show that an electrodialysis separation is not useful in a study of the physiological breakdown of Jonathan apples. This does not mean that such fruits are not measurably, as well as visibly, different from those that are normal. For instance, it was found (3) that they differed in catalase activity and in composition. Since physiological breakdown results in the disintegration and death of the cells it is probable that factors, such as respiration and electrical resistance, are affected. Possibly also a different rate of electrodialysis than that described here would show a differentiation.

Summary and conclusions

A comparative electrodialytic study of normal and physiologically broken down Jonathan apples revealed no essential difference between the two types of fruits.

Extracts obtained from the anode compartment consisted almost entirely of malic acid. Only traces or very small amounts of nitrate, sulphate, and chloride ions were found to be present.

Extracts taken from the cathode compartment contained very little calcium and appreciable amounts of potassium, enough to account for over half of the total ash.

Practically all of the basic dialyzable material was obtained during the first six-hour period. The malic acid continued to dialyze out, but at a decreasing rate, throughout three six-hour periods.

The electrodialysis of a malic acid-sugar solution was similar to that of apple tissue itself.

The construction of a three compartment cell from a rubber battery box is described.

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BRIEF PAPERS

CHANGES IN OSMOTIC PRESSURE OF BANANAS DURING RIPENING

(WITH TWO FIGURES)

Various data are found in the literature indicating an increase in moisture content of the pulp of bananas during ripening. Of the physiological processes occurring, respiration causes the production of water. At the same time water is utilized in the hydrolysis of starch to sugar. As these two processes occur simultaneously, a part of the water produced by respiration may be used up in the hydrolysis of starch. GORE[1] has calculated the water formed by respiration and that utilized in the hydrolysis of starch, and has found that except when the banana becomes over-ripe, the water formed in respiration does not equal that used in hydrolysis. He also points out the probable changes in osmotic pressure with consequent transfer of water from peel to pulp.

The present communication deals with quantitative measurements of osmotic pressures of bananas and other parts of the bunch during ripening.

Experimental

In table I each value represents a composite sample of two fingers selected at random from the middle hands of three bunches of fruit (Gros Michel from Jamaica), which had been ripened at 68° F. and 90–95 per cent. relative humidity, in specially constructed ripening rooms. Peel and pulp were separated, ground in a mortar, and the cell sap expressed by centrifuging. The freezing point of the sap was determined according to regular cryoscopic technique. Corrections for undercooling were made from the formula:[2]

$$\Delta = \Delta^1 - 0.0125\ U\ \Delta^1$$

where

Δ = corrected freezing point.
Δ^1 = observed freezing point.
U = degrees of undercooling below the observed freezing point.

From the corrected freezing point, the osmotic pressure, in atmospheres, was calculated from the formula:[2]

$$P = 12.06\ \Delta - 0.021\ \Delta^2$$

[1] Gore, H. C. Changes in composition of peel and pulp of ripening bananas. Jour. Agr. Res. 3: 187–203. 1914.

[2] Morrow, C. A. Biochemical laboratory methods. (p. 81). John Wiley and Sons. New York. 1927.

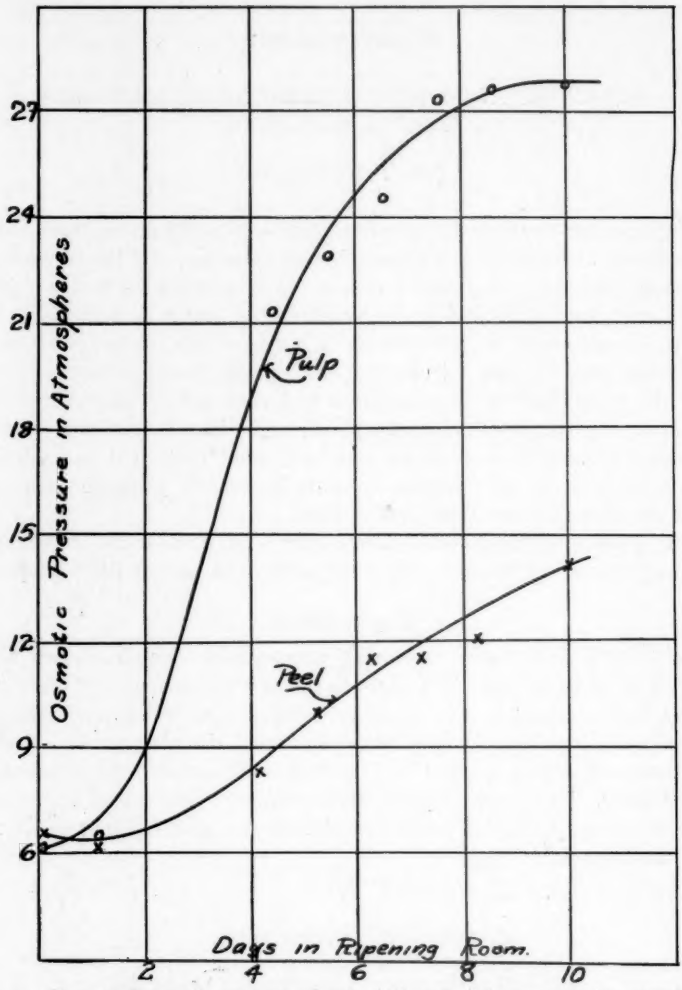


FIG. 1. Change of osmotic pressure of pulp and peel of bananas during ripening.

When two solutions of different osmotic pressures are separated by a semi-permeable membrane water moves from the region of less concentration of solute to that of the greater concentration of solute, with a tendency to produce equilibrium.

The results in table I show that there are marked changes in the osmotic pressure of the pulp and peel of the banana during ripening. At 0 days

(*i.e.* at the time of discharge from the boat at Boston) the osmotic pressures of pulp and peel are approximately equal and therefore at equilibrium, but as ripening progresses there is a marked increase of pressure in the pulp with a lesser increase in pressure of the peel. Differences in pressure between the peel and pulp naturally result in the migration of water from the former to the latter. These differences may be explained by the fact that while starch, the major constituent of the pulp of green bananas, does not affect the osmotic pressure to an appreciable extent, when this starch is hydrolyzed to sugar during the ripening process, the osmotic pressure then increases rapidly because of and in proportion to this production of sugar. The total sugars in the pulp amount to as much as 21 per cent. (unpublished data), while the sugar content of the peel is much lower with a maximum of about 4.4 per cent.

During the ripening period the fruit showed a loss in weight of 2.44 per cent. Furthermore the pulp increased from 68.5 per cent. of the total weight before ripening to 70.9 per cent. of the total weight after ripening, a gain of 2.4 per cent. GORE (*loc. cit.*) cites corresponding gains of from 2.24 to 3.90 per cent.

TABLE I

OSMOTIC PRESSURE OF PULP AND PEEL OF BANANAS DURING RIPENING

DAYS IN RIPENING ROOM	COLOR OF PEEL	OSMOTIC PRESSURE			
		PULP*		PEEL*	
		I atm.	*II* atm.	*I* atm.	*II* atm.
0	Green	6.19	6.33	6.57	6.63
1	Starting to turn yellow	6.66	6.69	6.31	6.30
4	Very slight green	21.04	21.28	8.46	8.34
5	Green tip	23.70		10.38	
6	Full yellow	24.60		11.56	
7	Full yellow	27.40		11.56	
8	Speckled	27.87		12.39	
10	Speckled, fingers drop	27.88		14.68	

* Duplicate determinations were discontinued after the fourth day as the variation due to technique was shown to be negligible. (See figure 1 for graphical representation of above data.)

TABLE II

OSMOTIC PRESSURES OF DIFFERENT PARTS OF THE BANANA FRUIT BUNCH DURING RIPENING

DAYS IN RIPENING ROOM	COLOR	OSMOTIC PRESSURE				
		PULP	PEEL	NECK	CROWN	STALK
		atm.	*atm.*	*atm.*	*atm.*	*atm.*
0	Green	7.15	6.19	7.17	7.82	6.81
3	Very slight green	16.91	9.29	7.41	6.55	6.10
6	Green tip	25.48	10.47	6.80	7.02	5.36
9	Slightly speckled	29.06	12.42	6.46	7.00	5.58

Commercial shrinkage studies have shown that cut hands suffered slightly greater shrinkage than hands ripened on the stalk. Our assumption was that a transfer of water from the stalk to the hands took place. In table II are presented our data from Gros Michel fruit (from Tela,

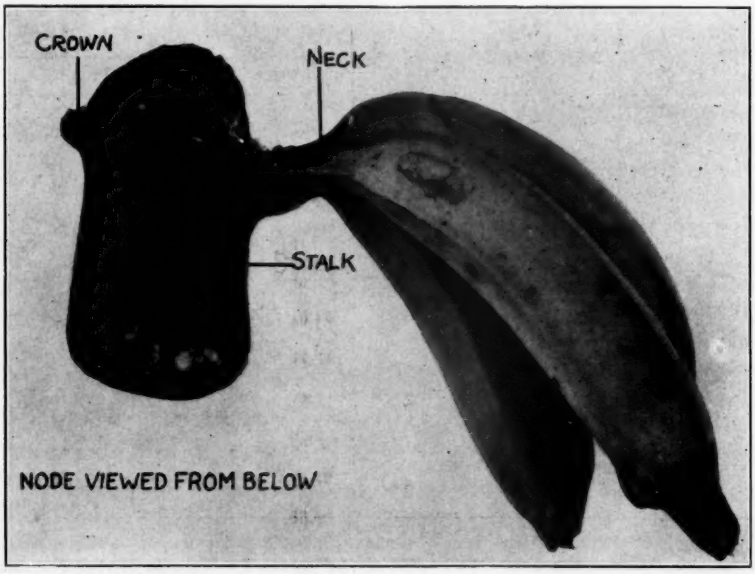


FIG. 2. Banana node, indicating parts tested in table II.

Honduras) ripened at 68° F., 90–95 per cent. relative humidity. Each value represents a composite sample from seven fingers, the necks of the corresponding hand (17 fingers), the corresponding crown, and a section of the stalk at the crown (fig. 2). The samples were crushed in a mortar, centrifuged, and filtered when necessary, to give a clear sap.

The results show that as ripening progresses there is, in general, a gradual decrease in order of osmotic pressures from the pulp to the stalk, indicating a transfer of water from other parts of the bunch of fruit to the pulp.

Summary and conclusions

Osmotic pressure determinations of different parts of the bunch during ripening of bananas indicate that changes of pressure are such as to bring about a transfer of water from peel to pulp, as well as from the stalk through the crown and neck to the peel and pulp.—FRANK C. STRATTON and HARRY VON LOESECKE, *Research Laboratories, United Fruit Company, Boston, Mass.*

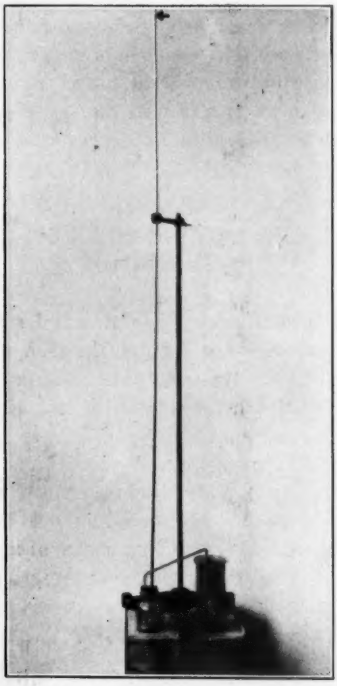
A QUANTITATIVE DEMONSTRATION OF OSMOTIC EQUILIBRIUM

Demonstrations of the diffusion of solutes and solvents through differentially permeable membranes are common in all branches of natural and biological science. Ordinarily the membrane used is so imperfect, however, that most of the value of the demonstration is lost and the students do not obtain a concept of osmotic pressure as a diffusion equilibrium dependent upon the relative activity of solute and solvent on the two sides of the membrane. The following modification of the PFEFFER technique for measuring osmotic pressure has been used in our laboratory for several years. It has the advantage of requiring no special equipment or technique to give quantitative osmotic pressures for sucrose solutions at concentrations between 0.01 and 0.10 molar, with an accuracy within two to four per cent. of the calculated values by the FINDLAY or other corrected equations.

The apparatus is set up as shown in the figure with a reservoir manometer and a copper ferrocyanide membrane deposited within the walls of a Livingston cylindrical atmometer cell. The primary difficulties are the maintenance of an unbroken copper membrane on the *inside* of the porous cup, and adequate wiring or clamping of the rubber stoppers to withstand the pressures developed.

The membrane is formed by drying a new, unshellacked atmometer cylinder at 100° C. An old cylinder may be used if the shellac is removed

and it is cleaned with ammonia and nitric acid and washed for several hours in running water. The dry tube is covered with 0.5 M copper sulphate solution and the air removed by repeated evacuation. The saturated tube is then quickly wiped inside and out with a clean cloth and filled within one-fourth inch of the top with 0.25 M potassium ferrocyanide solution which also contains the desired concentration of sucrose. The two solutes should be mixed and made up to volume together. The ferrocyanide solution should not come in contact with the outside of the cell, for a double membrane will be formed which may develop defects. A



rubber stopper with a piece of capillary glass tubing to connect the cell to the manometer is inserted and carefully wired into the cell. A metal washer slightly smaller than the stopper will prevent the wires being forced into the rubber. Special screw clamps may be used for holding the stopper but they are more likely to break the cell than is the wire. If a soft iron or copper wire of medium size is used it may be cut into short lengths and five to ten of these laid fanshaped over the top and bent down

past the shoulder of the cell on both sides. The ends of these wires are then tied in under the shoulder, drawn tight, bent up and tied a second time. The cell and tube should be filled with solution, although small air bubbles will do no harm.

A small quantity of mercury is placed in the bottom of the reservoir, the bottle filled with water and a two-holed stopper, containing the cell connection and manometer, wired in. The osmotic cell is then completely immersed in 0.5 M copper sulphate solution and, as soon as diffusion starts, mercury is added to the manometer tube until equilibrium is reached. A 4 to 6 mm. manometer tube should be used so that mercury can be added from the top. This avoids dilution of the test solution through inward diffusion of water.

Five-tenths molar copper sulphate is roughly isotonic with 0.25 M potassium ferrocyanide, but for accurate work a blank determination is made and subtracted from the readings for the test solutions. The presence of the salts insures a good membrane and the ferrocyanide also acts as a preservative for the sucrose.

The setting up of the apparatus requires some manipulative skill, but pressures of an atmosphere are demonstrated readily and pressures up to two and one-half atmospheres have been produced in our laboratory. Properly constructed membranes are so slowly permeable to sucrose as to hold 80 per cent. of the maximum pressure after three months.—W. E. Loomis, *Department of Botany, Iowa State College, Ames, Iowa.*

NOTES

Pasadena Meeting.—The American Association for the Advancement of Science holds a summer meeting at the California Institute of Technology, in Pasadena, June 15–20, 1931. The American Society of Plant Physiologists will participate in this meeting to a limited extent. A program is being arranged under the leadership of Dr. D. R. HOAGLAND, of the University of California. The attention of our western members is directed to this opportunity for mutual encouragement, and for the discussion of progress in research. These summer meetings may be somewhat more local in character than the winter meetings, but they will provide opportunities for less expensive attendance at the meetings for those who happen to reside within a short distance of the meeting places chosen. It is hoped that the western group of members will respond cordially to the efforts of the program committee for this summer gathering.

Council Representatives.—President H. R. KRAYBILL has appointed Dr. C. A. SHULL, of the University of Chicago, and Dr. GEORGE J. PEIRCE, of Stanford University, to represent the American Society of Plant Physiologists on the Council of the American Association for the Advancement of Science, during the coming year.

Memorial Committee.—At the Cleveland meeting of the Society it was decided to appoint a committee to arrange for special features of our annual meetings, with special reference to the commemoration of anniversaries of the great leaders in the field of plant physiology during past epochs. The Cleveland meeting was dedicated to JAN INGEN-HOUSZ, who was born December 8, 1730. It would have been very appropriate to have had a more elaborate program in memory of the 200th anniversary of his birth. It was felt that future events of this kind might be commemorated by special meetings devoted to the different phases of influence which these men have exerted upon the development of plant physiology in their own time and ours. It was also decided that the meeting of 1932 should be made commemorative of the life of JULIUS VON SACHS, the 100th anniversary of whose birth occurs in that year.

The Memorial Committee provided for has now been appointed by the President of the Society as follows: Dr. C. F. HOTTES, University of Illinois, chairman; Dr. C. O. APPLEMAN, University of Maryland; and Dr. FRANK M. ANDREWS, Indiana University.

Program Committee.—President KRAYBILL has also appointed the Program Committee for the New Orleans Meeting in December, 1931. The members chosen for this service are Dr. E. S. REYNOLDS, the Missouri

Botanic Garden and Washington University, chairman; Dr. D. B. ANDERSON, North Carolina State College; Dr. L. J. PESSIN, Southern Forest Experiment Station; and Dr. C. B. LIPMAN, University of California. The Secretary, Dr. W. A. GARDNER, Alabama Polytechnic Institute, is also *ex officio* a member of the program committee.

Membership Committee.—The Membership Committee appointed to serve during the ensuing year will appreciate the cooperation of all members in their efforts to reach all of those who need the literature of plant physiology in their work. Dr. R. E. GIRTON, of Purdue University, is chairman of the committee, and serving with him are Dr. J. P. BENNETT, University of California, and Dr. LEE HUTCHINS, of the Bureau of Plant Industry, U. S. D. A., Washington, D. C. All applications for membership receive prompt attention from the Executive Committee.

Purdue University Section.—During the last year the Purdue Section of the American Society of Plant Physiologists has had its best year so far, a membership of 38, and an average attendance of 29. The meetings occur at 4:00 P. M. in Stanley Coulter Hall. The 1930–1931 program will be interesting to all of our members. It shows what an active group can do with the privileges of sectional organization. The entire program consisted of 13 meetings, with the following addresses:

October 14, (Dinner meeting) The Fifth International Botanical Congress, by Dr. J. C. ARTHUR

October 20, Effect of mineral nutrition on development of rusts and mildews, by K. D. DOAK

November 3, The equipment and arrangement of the plant physiology laboratory for teaching and research, by R. E. GIRTON

November 17, Some interesting phases of insect metabolism, by W. A. HIESTAND

December 1, Country life in Scotland, Prof. AITKENHEAD

December 15, Laboratory versus field germination tests in soybeans, by G. H. CUTLER

January 5, 1931, Reports on the Cleveland meetings of the A. A. A. S., a joint evening meeting with the Biological Society

January 19, Light and some of its effects upon plants, by G. E. READ

February 2, Factors affecting hardiness in peach buds, F. P. CULLINAN

February 16, A physiological consideration of the ontogeny of leaf, root and stem, by E. J. KOHL

March 2, Bacterial decomposition of cellulose at high temperatures, by P. A. TETRAULT

March 16, Protein synthesis in plants, by H. R. KRAYBILL

April 6, (Dinner meeting). Dr. E. N. TRANSEAU, Ohio State University

Such meetings must prove to be helpful and stimulating in the development of a high grade research atmosphere in an institution. The example is worthy of emulation by others. At the meeting on March 2, officers for 1931–1932 were elected. Chairman, Dr. LAURENZ GREENE; Secretary, Dr. E. J. KOHL.

Agricultural Books.—Baillière, Tindall and Cox, 7 and 8 Henrietta St., Covent Garden, London, W. C. 2, offer their services to any of our members who may have need of Agricultural Books, either old or new. They will send their catalog to any one who may be interested in acquiring works of this type, and will give courteous and prompt attention to all orders which may be sent to them.

Portraits.—We are fortunate to be able to present in this number a portrait of Dr. F. F. BLACKMAN, of the Botany School, Cambridge, from a crayon of unusual merit by one of Dr. BLACKMAN'S students, Miss DE BIDEN FOOTNER, in July, 1924. All portraits are obtainable from the editor at Chicago at 12 cents each, or in complete sets of 13 for $1.45 in postage. The number printed is limited, as the demand has not been large. Laboratories should avail themselves of the opportunity to obtain these prints before any of them become exhausted.

Collected Works of Emile Godlewski.—The Academie Polonaise des Sciences et des Lettres has published Vol. I of the collected works of EMILE GODLEWSKI, père, the beginning of a three volume edition of his complete works. This publication was undertaken to celebrate GODLEWSKI'S 80th birthday. The value of GODLEWSKI'S work to the fields of agronomy, agriculture, and plant physiology makes this collection of his contributions unusually worth while. The first volume contains 599 pages, and covers the period from 1870–1890. The second volume will contain the papers appearing from 1891 to 1910; and the last volume, the recent work, since 1910, particularly his popularizations of agricultural science. The papers are printed in the original language, but where printed only in Polish, summaries in other languages, or translations, will be provided. The first volume has a fine portrait of GODLEWSKI, and a facsimile reproduction of a page from one of his manuscripts, a plant physiology which has not yet been published. There is also an autobiography in volume I, and the last volume will have a bibliographical index of his complete works. These three volumes will be a splendid monument to GODLEWSKI'S industry and achievement in research. Inquiries for the work should be addressed to the Academie Polonaise des Sciences et des Lettres, 17 Rue Slawkowska, Cracovie, Poland.

Handbook of Plant Nutrition.—A voluminous work in two volumes in this field is announced by Julius Springer, Linksstrasse 23–24, Berlin W9. Its title is Handbuch der Pflanzenernährung und Düngerlehre, and volume II, Düngemittel und Düngung has come out ahead of volume I, Pflanzenernährung, which will appear later in the spring of 1931. The editor of the work is Dr. F. Honcamp, Director of the Experiment Station at Rostock, who has had the assistance of 50 cooperators in producing the work. The material of volume II is presented in nine chapters, the first of which outlines the historical background, and the general influence of fertilizers on soils and plants. The next three chapters deal with the natural and artificial fertilizers, and their application. Chapter five gives an account of fertilizer practice with specific crop plants, some 25 different agricultural species being considered in detail. The later chapters discuss forestry fertilizer problems, fertilization of moor and heath soils, ponds, and the use of fertilizer materials in control of disease, animal pests, and weeds. With the index, it is a volume of 919 pages. The price of volume II in paper cover is 86 RM, and with cloth binding 89.8 RM. It seems to be a valuable addition to the literature of nutrition of agricultural plants.

Principles of Agrobiology.—This little book, by Oswin W. Wilcox, claims to be the only work which "gives an orderly and adequate statement of the axiomatic fundamentals of crop growth." It is also claimed that agriculture can now be, in the hands of the ablest practitioners, an exact art. The booklet contains less than a hundred pages. Part I presents a discussion of the primary laws of agrobiology, upon which agriculture as an exact art, is predicated. These are: Law of the constancy of type, law of definite growth powers, law of the universality of essential growth factors, law of the constancy of action of growth factors, law of the joint action of growth factors, law of definite optima, law of diminishing increments of yield, law of increasing increments of yield, the concentration law of growth factors, and the logistic law of crop growth. Part II considers the general law of growth factors and its derivatives; and part III, fertilizer statics. There is a brief mathematical appendix.

The author has copyrighted his ideas, which will militate against any general use of them. The book is published by the Palmer Publishing Corporation, New York. The price is $4.00 per copy.